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(57) Abstract

The present invention relates to an isolated protein or polypeptide corresponding to a coat protein or other polypeptide of a grapevine leafroll virus. The encoding DNA molecule either alone in isolated form or in an expression system, a host cell, or a transgenic grape plant is also disclosed. Another aspect of the present invention relates to a method of imparting grapevine leafroll resistance to grape plants by transforming them with the DNA molecule of the present invention. A method for imparting tristeza virus resistance in citrus plants using the DNA molecule of the present invention is also disclosed.

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GRAPEVINE LEAFROLL VIRUS PROTEINS AND THEIR USES

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FIELD OF THE INVENTION

The present invention relates to grapevine leafroll virus proteins, DNA molecules encoding these proteins, and their uses.

BACKGROUND OF THE INVENTION

The world's most widely grown fruit crop, the grape (*Vitis sp.*), is cultivated on all continents except Antarctica. Major grape production centers are in European countries (including Italy, Spain, and France), which constitute about 70% of the world grape production (Mullins et al., Biology of the Grapevine, Cambridge, U.K., University Press (1992)). The United States is the eighth largest grape grower in the world. Although grapes have many uses, a major portion of grape production (~80%) is used for wine production. Unlike cereal crops, most of the world's vineyards are planted with traditional grapevine cultivars, which have been perpetuated for centuries by vegetative propagation. Several important grapevine virus and virus-like diseases, such as grapevine leafroll, corky bark, and Rupestris stem pitting, are transmitted and spread through the use of infected vegetatively propagated materials. Thus, propagation of certified, virus-free materials is one of the most important disease control measures. Traditional breeding for disease resistance is difficult due to the highly heterozygous nature and outcrossing behavior of grapevines,

and due to polygenic patterns of inheritance. Moreover, introduction of a new cultivar may be prohibited by custom or law. Recent biotechnology developments have made possible the introduction of special traits, such as disease resistance, into an established cultivar without altering its horticultural characteristics.

Many plant pathogens, such as fungi, bacteria, phytoplasmas, viruses, and nematodes can infect grapes, and the resultant diseases can cause substantial losses in production (Pearson et al., Compendium of Grape Diseases, American Phytopathological Society Press (1988)). Among these, viral diseases constitute a major hindrance to profitable growing of grapevines. About 34 viruses have been isolated and characterized from grapevines. The major virus diseases are grouped into: (1) the grapevine degeneration caused by the fanleaf nepovirus, other European nepoviruses, and American nepoviruses, (2) the leafroll complex, and (3) the rugose wood complex (Martelli, ed., Graft Transmissible Diseases of Grapevines, Handbook for Detection and Diagnosis, FAO, UN, Rome, Italy (1993)).

Grapevine leafroll complex is the most widely distributed of the major diseases of grapes. According to Goheen (Goheen, "Grape Leafroll," in Frazier et al., eds., Virus Diseases of Small Fruits and Grapevines (A Handbook), University of California, Division of Agricultural Sciences, Berkeley, Calif, USA, pp. 209-212 (1970), grapevine leafroll-like disease was described as early as the 1850s in German and French literature. The viral nature of the disease and graft transmission were first demonstrated by Scheu (Scheu, D. D. Weinbau 14:222-358 (1935). In 1946, Harmon and Snyder (Harmon et al., Proc. Am. Soc. Hort. Sci. 74:190-194 (1946)) determined the virus nature of White Emperor disease in California. It was later proven by Goheen et al. (Goheen et al., Phytopathology, 48:51-54 (1958)) that both leafroll and "White Emperor" diseases were the same, and only the name "leafroll" was retained.

Leafroll is a serious virus disease of grapes and occurs wherever grapes are grown. This wide distribution of the disease has come about through the propagation of diseased vines. It affects almost all cultivated and rootstock varieties of *Vitis*. Although the disease is not lethal, it causes yield losses and reduced sugar content. Scheu estimated in 1936 that 80 per cent of all grapevines planted in Germany were infected (Scheu, Mein Winzerbuch, Berlin, Reichsnahrstand-Verlags (1936)). In many California wine grape vineyards, the incidence of leafroll (based on a 1959 survey of field symptoms) agrees with Scheu's initial observation in German vineyards (Goheen et al., Amer. J. Enol. Vitic., 10:78-84 (1959)). The current situation on leafroll disease appears similar (Goheen, The American Phytopathological Society, St. Paul, Minnesota:APS Press, 1:47-54 (1988). Goheen estimated that the disease causes an annual loss of about 5-20 per cent of the total grape production (Goheen (1970); Goheen (1988)). The amount of sugar in individual berries of infected vines is only about 1/2 to 2/3 that of berries from noninfected vines (Goheen (1958)).

Symptoms of leafroll disease vary considerably depending upon the cultivar, environment, and time of the year. On red or dark-colored fruit varieties, the typical downward rolling and interveinal reddening of basal, mature leaves is prevalent in autumn and is less apparent in spring or early summer. On light-colored fruit varieties, symptoms are less conspicuous, usually downward rolling accompanied by interveinal chlorosis. Moreover, many infected rootstock cultivars do not develop symptoms. In these cases, the disease is usually diagnosed with a woody indicator indexing assay using *Vitis vivifera* cv. Carbernet Franc (Goheen (1988)).

Ever since Scheu demonstrated that leafroll was graft transmissible, a virus etiology has been suspected (Scheu (1935)). Several virus particle types have been isolated from leafroll diseased vines. These include potyvirus-like (Tanne et al., Phytopathology, 67:442-447 (1977)), isometric virus-

like (Castellano et al., Vitis, 22:23-39 (1983)) and Namba et al., Ann. Phytopathol. Soc. Japan, 45:70-73 (1979)), and closterovirus-like (Namba, Ann. Phytopathol. Soc. Japan, 45:497-502 (1979)) particles. In recent years, however, long flexuous closteroviruses ranging from 1,400 to 2,200 nm in length have been most consistently associated with leafroll disease (Castellano (1983), Faoro et al., Riv. Patol. Veg., Ser IV, 17:183-189 (1981); Gugerli et al., Rev. Suisse Viticult. Arboricult. Hort., 16:299-304 (1984); Hu et al., J. Phytopathol., 128:1-14 (1990); Milne et al., Phytopathol. Z., 110:360-368 (1984); Zee et al., Phytopathology, 77:1427-1434 (1987); Zimmermann et al., J. Phytopathol., 130:205-218 (1990). These closteroviruses are referred to as grapevine leafroll associated viruses (GLRaV). At least six serologically distinct types of GLRaV's (GLRaV-1 to -6) have been detected from leafroll diseased vines (Table 1) (Boscia et al., Vitis, 34:171-175 (1995); (Martelli, "Leafroll," pp. 37-44 in Martelli, ed., Graft Transmissible Diseases of Grapevines, Handbook for Detection and Diagnosis, FAO, Rome Italy, (1993)). The first five of these were confirmed in the 10th Meeting of the International Council for the Study of Virus and Virus Diseases of the Grapevine (ICVG) (Volos, Greece, 1990). Through the use of monoclonal antibodies, however, the original GLRaV II described in Gugerli (1984) has been shown to be an apparent mixture of at least two components, IIa and IIb (Gugerli et al., "Grapevine Leafroll Associated Virus II Analyzed by Monoclonal Antibodies," 11th Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine, Montreux, Switzerland, pp. 23-24 (1993)). Recent investigation with comparative serological assays (Boscia (1995)) demonstrated that the IIb component of cv. Chasselas 8/22 is the same as the GLRaV-2 isolate from France (Zimmermann (1990)) which also include the isolates of grapevine corky bark associated closteroviruses from Italy (GCBaV-BA) (Boscia (1995)) and from the United States (GCBaV-NY) (Namba et al., Phytopathology, 81:964-970 (1991)). The IIa component of cv. Chasselas 8/22 was given

the provisional name of grapevine leafroll associated virus 6 (GLRaV-6). Furthermore, the antiserum to the CA-5 isolate of GLRaV-2 produced by Boscia et al. (Boscia et al., Phytopathology, 80:117 (1990)) was shown to contain antibodies to both GLRaV-2 and GLRaV-1, with a prevalence of the latter (Boscia (1995)).

Several shorter closteroviruses (particle length 800 nm long) have also been isolated from grapevines. One of these, called grapevine virus A (GVA) has also been found associated, though inconsistently, with the leafroll disease (Agran et al., Vitis, 29:43-48 (1990); Conti, et al., Phytopathol. Mediterr., 24:110-113 (1985); Conti et al., Phytopathology, 70:394-399 (1980)). The etiology of GVA is not really known; however, it appears to be more consistently associated with rugose wood *sensu lato* (Rosciglione at al., Rev. Suisse Vitic Arboric. Hortic., 18:207-211 (1986); and Zimmermann (1990)). Another short closterovirus (800 nm long) named grapevine virus B (GVB) has been isolated and characterized from corky bark-affected vines (Boscia et al., Arch. Virol., 130:109-120 (1993); Namba (1991)).

As suggested by Martelli, leafroll symptoms may be induced by more than one virus or they may be simply a general plant physiological response to invasion by an array of phloem-inhabiting viruses. Grapevine leafroll is induced by one (or a complex) of long closteroviruses (particle length 1,400 to 2,200 nm).

Grapevine leafroll is transmitted primarily by contaminated scions and rootstocks. However, under field conditions, several species of mealybugs have been shown vectors of leafroll (Engelbrecht et al., Phytophylactica, 22:341-346 (1990); Engelbrecht et al., Phytophylactica, 22:347-354 (1990); Rosciglione, et al., (Abstract), Phytoparasitica, 17:63-63 (1989); and Tanne, Phytoparasitica, 16:288 (1988)). Natural spread of leafroll by insect vectors is rapid in various parts of the world. In New Zealand, observations of three vineyards showed that the number of infected vines nearly doubled in a single year (Jordan et al.,

11th Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine, Montreux, Switzerland, pp. 113-114 (1993)). One vineyard became 90% infected 5 years after GLRaV-3 was first observed. Prevalence of leafroll worldwide may increase as chemical control of mealybugs becomes more difficult due to the unavailability of effective insecticides.

In view of the serious risk grapevine leafroll virus poses to vineyards and the absence of an effective treatment, there is a need to prevent this disease and the resulting economic losses. The present invention overcomes this deficiency in the art.

SUMMARY OF INVENTION

The present invention relates to an isolated protein or polypeptide corresponding to a protein or polypeptide of a grapevine leafroll virus. The encoding RNA and DNA molecules, in either isolated form or incorporated in an expression system, vectors, host cells, and transgenic *Vitis* or citrus scions or rootstock cultivars, are also disclosed.

Another aspect of the present invention relates to a method of imparting grapevine leafroll virus resistance to *Vitis* scion or rootstock cultivars by transforming them with a DNA molecule encoding a protein or polypeptide of a grapevine leafroll virus. These DNA molecules can also be used in transformation of citrus scion or rootstock cultivar to impart tristeza virus resistance to such cultivars.

The present invention also relates to an antibody or binding portion thereof or probe which recognizes the protein or polypeptide or the nucleic acid encoding same.

Grapevine leafroll virus resistant transgenic variants of the current commercial grape cultivars and rootstocks allows for improved control of the virus while retaining the varietal characteristics of specific cultivars. Furthermore, these variants permit control of GLRaV transmitted by contaminated scions or rootstocks or by GLRaV-carrying mealybugs or other

insect pests. With respect to the latter mode of transmission, the present invention circumvents increasingly restricted pesticide use, which has made chemical control of mealybug infestations increasingly difficult. Thus, the interests of the environment and the economics of grape cultivation and wine making are benefited by the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B, illustrates the results of Northern blot hybridization. Figure 1B shows that probe made from a clone insert gave positive reaction with itself (lane 3) and to dsRNA from leafroll infected tissues (lane 1), but not with nucleic acids extracted from healthy grapevines (lane 2). Lane M contains molecular weight markers (HindIII digested lambda DNA). Figure 1A depicts an ethidium bromide stained agarose gel before transfer to a membrane.

Figure 2 presents an analysis of GLRaV-3 dsRNA by electrophoresis on an ethidium bromide stained agarose gel. A dsRNA of ca. 16 kb was readily isolated from diseased grapevine (lane 6), but not from the healthy control (lane 5). Other samples that were used for control were tobacco mosaic virus dsRNA (lane 1); cucumber mosaic virus dsRNA (lane 2); pBluescript vector (lane 3) and an insert of clone pC4. λ *Hin*dIII digested lambda DNA was used as molecular weight markers (lane M).

Figure 3 is a Western blot of antibodies to GLRaV-3 that reacted to proteins produced by cDNA clones after IPTG induction in *E. coli*. Similar banding patterns were observed whether a polyclonal (panel A) or a monoclonal antibody (panel B) was used. Lane 1 shows clone pCP10-1; lane 2, pCP5; lane 3, pCP8-4; and lane 4, the native coat protein from GLRaV-3 infected tissue. Lane M is a prestained protein molecular weight marker.

Figure 6 shows the cDNA clones containing the coding region for the coat protein of the NY1 isolate of GLRaV-3. Three clones (pCP8-4, pCP5, pCP10-1) were identified by

immunoscreening a cDNA library prepared in lambda ZAP II. Two other clones were aligned after plaque hybridization and nucleotide sequencing. The coat protein ORF is shown by an arrow in an open rectangle.

Figure 5 is the phylogenetic tree generated using results obtained using the Clustal Method of MegAlign program in DNASTAR for the coat protein of GLRaV-3. The coat protein of GLRaV-3 was incorporated into a previously described alignment (Dolja et al., Ann. Rev. Phytopathol., 32:261-285 (1994)) for comparison. The other virus sequences were obtained from current databases: apple chlorotic leafspot virus (ACLSV); apple stem grooving virus (ASGV); apple stem pitting virus (ASPV); barley yellow mosaic virus (BaMV); beet yellows closterovirus (BYV); diverged copies of BYV and CTV coat proteins (BYV p24 and CTV p27, respectively); citrus tristeza virus (CTV); grapevine virus A (GVA); grapevine virus B (GVB); lily symptomless virus (LSV); lily virus X (LVX); narcissus mosaic virus (NMV); pepper mottle virus (PeMV); papaya mosaic virus (PMV); potato virus T (PVT); potato virus S (PVS); potato virus M (PVM); potato virus X (PVX); tobacco etch virus (TEV); tobacco vein mottle virus (TVMV); and white clover mosaic virus (WcMV).

Figure 6 depicts an analysis of reverse transcription polymerase chain reaction (RT-PCR) to detect GLRaV-3 in a partially purified virus preparation. The original sample concentration is equivalent to 50 mg/μl of phloem tissue (lane 1) which was diluted by 10-fold series as 10^{-1} (lane 2), 10^{-2} (lane 3), 10^{-3} (lane 4), 10^{-4} (lane 5), and 10^{-5} (lane 6), respectively. The expected 219 bp PCR product was clearly observed up to lane 4, which is equivalent to a detection limit of 10 μg of phloem tissue. Lane 7 was a healthy control. Lane 8 was dsRNA for positive control. Lanes 9-11 were also used for positive controls of purified viral RNA (lane 9), dsRNA (lane 10), and plasmid DNA (pC4) (lane 11) as templates, respectively. Lane M contains molecular weight markers (*Hae*III digested fX 174 DNA).

Figures 7A-7B depicts comparative analysis of Nested PCR with immuno-capture preparations on field collected samples. Using a polyclonal antibody to GLRaV-3 for immuno-capture, the expected 648 bp PCR product was not consistently observed in the first round of PCR amplification with external primers over a range of samples (lanes 1-7, Figure 7A). However, the expected 219 bp PCR product amplified by internal primers was consistently observed over all seven samples (lanes 1-7, Figure 7B). A similar inconsistency is also shown in a sample prepared by proteinase K-treated crude extract (compare panels A to B on lane 8). With dsRNA as template, the expected PCR products were readily observed in both reactions (compare lane 10 in Figure 7A and 7B). No such products were observed on a healthy sample (lane 9). Lane M contained molecular weight markers (*HaeIII* digested fX 174 DNA).

Figures 8A-8B depict comparative studies on the sensitivity of Nested PCR with samples prepared using proteinase K-treated crude extract (Figure 8A, PK Nested PCR) and by immuno-capture preparation (Figure 8B, IC Nested PCR). Nested PCR was performed on samples with serial 10-fold dilutions of up to 10^{-6} in a proteinase K-treated and 10^{-4} in an immuno-capture preparation. The expected 219 bp PCR product was observed up to 10^{-5} in PK Nested PCR and over 10^{-6} (the highest dilution used in this test) in IC Nested PCR. A similar PCR product was also observed with dsRNA template but not healthy grape tissues (H. CK). Lane M contained molecular weight markers (*HaeIII* digested fX 174 DNA).

Figure 9 shows partial genome organization for GLRaV-3 and the cDNA clones used to determine nucleotide sequence. Numbered lines represent nucleotide coordinates in kilobases (kb).

Figure 10 depicts the proposed genome organization of the GLRaV-3 in comparison with three other closterovirus genomes, BYV, CTV, and LIYV (Dolja (1994)). Homologous proteins are shown by identical patterns. Papain-like proteinase (P-PRO); methyltransferase of type 1 (MTR1); RNA helicase of superfamily 1 (HEL1); RNA polymerase of supergroup 3 (PLO3);

HSP70-related protein (HSP70r); and capsid protein forming filamentous virus particle (CPf).

Figure 11 is the phylogenetic tree showing the amino acid sequence relationship of the helicase of alphaviruses. The helicase domain of GLRaV-3 (291 aa) from the present study is used. The other virus sequences were obtained from current databases (Swiss-Prot and GenBank, release 84.0). Apple chlorotic leafspot virus (ACLSV); broad bean mottle virus (BbMV); brome mosaic virus (BMV); beet yellow closterovirus (BYV); cowpea chlorotic mottle virus (CcMV); cucumber mosaic virus (CMV); fox mosaic virus (FxMV); lily symptomless virus (LSV); lily virus X (LXV); narcissus mosaic virus (NMV); pea early browning virus (PeBV); papaya mosaic virus (PMV); poplar mosaic virus (PopMV); peanut stunt virus (PSV); potato virus S (PVS); potato virus M (PVM); potato virus X (PVX); strawberry mild yellow edge-associated virus (Sm Yea V); tomato aspermy virus (TAV); tobacco mosaic virus (TMV); tobacco rattle virus (TRV); and white clover mosaic virus (WcMV).

Figure 12 shows the phylogenetic tree for the RNA dependent RNA polymerases (RdRp) of the alpha-like supergroup of positive strand RNA viruses. The deduced amino acid sequence of the RdRp of GLRaV-3 was incorporated into a previously described alignment (Dolja (1994)) for comparison. The other virus sequences were obtained from current databases: Apple chlorotic leafspot virus (ACLSV); alfalfa mosaic virus (AlMV); apple stem grooving virus (ASGV); brome mosaic virus (BMV); beet necrotic yellow vein virus (BNYVV); beet yellow virus (BYV); barley stripe mosaic virus (BSMV); beet yellow stunt virus (BYSV); cucumber mosaic virus (CMV); citrus tristeza virus (CTV); hepatitis E virus (HEV); potato virus M (PVM); potato virus X (PVX); raspberry bushy dwarf virus (RBDV); shallot virus X (SHVX); Sinbis virus (SNBV); tobacco mosaic virus (TMV); tobacco rattle virus (TRV); and turnip yellow mosaic virus (TYMV).

Figure 13 is the predicted phylogenetic relationship for viral and cellular HSP70 proteins. HSP70-related protein of GLRaV-3 (p59) was incorporated into a previously described

alignment (Dolja (1994)) for comparison. The sequences of BYV, CTV, and LIYV proteins were from Agranovsky et al., J. Gen. Virol., 217:603-610 (1991), Pappu et al., Virology, 199:35-46 (1994), and Klaassen et al., Virology, 208:99-110 (1995), respectively. Only the N-terminal half of beet yellow stunt virus HSP70-related protein (Karasev et al., J. Gen. Virol., 75:1415-1422 (1994)) is used. Other sequences were obtained from the Swiss-Prot database; their accession numbers are as follows: DNA1_BACSU, *Bacillus subtilis* (P13343); DNAK_ECOLI, *Escherichia coli* (P04475); HS70_CHICK (P08106); HS70_ONCMY, *Oncorhynchus mykiss* (P08108); HS70_PLACB, *Plasmodium cynomolgi* (Q05746); HS70_SCHMA, *Schistosoma mansoni* (P08418); HS70_XENLA, *Xenopus laevis* (P02827); HS71_DROME, *Drosophila melanogaster* (P02825); HS71_HUMAN (P08107); HS71_MOUSE (P17879); HS71_PIG (P34930); HS74_PARLI, *Paracentrotus lividus* (Q06248); HS74_TRYBB, *Trypanosoma brucei* (P11145); and ZMHSP702, maize gene for heat shock protein 70 exon 2 (X03697).

Figure 14 summarizes the strategies employed in the construction of the plant transformation vector pBin19GLRaV-3hsp90-12-3. A plant expression cassette, in the HindIII-EcoRI fragment containing CaMV 35S-35S promoters-AMV 5' untranslated sequence-43K ORF-Nos 3' untranslated region, was excised from pBI525GLRaV-3hsp90 and cloned into the similarly (restriction enzyme) treated plant transformation vector pBin19. The resulting clone, pBin19GLRaV-3hsp90-12-3, is shown. Locations of important genetic elements within the binary plasmid are indicated: BR, right border; BL, left border; Nos-NPT II, plant expressible neomycin phosphotransferase gene; Lac-LAC Z, plant expressible Lac Z gene; and Bacterial Kan, bacterial kanamycin resistance gene.

Figure 15 shows the *Agrobacterium*-binary vector pGA482G/cpGLRaV-3, which was constructed by cloning the HindIII fragment of pEPT8cpGLRaV-3 into a derivative of pGA482 and used for transformation via *Agrobacterium* or Biolistic approach.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to isolated DNA molecules encoding the proteins or polypeptides of a grapevine leafroll virus. A substantial portion of the grapevine leafroll virus genome, within which are a plurality of open reading frames, has been sequenced by the present inventors. One such DNA molecule contains an open reading frame encoding grapevine leafroll virus helicase and comprising the nucleotide sequence corresponding to SEQ ID NO:1. The helicase has an amino acid sequence corresponding to SEQ ID NO:2 and a molecular weight from about 146 to about 151 kDa, preferably about 148.5 kDa.

Another such DNA molecule comprises an open reading frame which codes for a grapevine leafroll virus RNA-dependent RNA polymerase and comprises the nucleotide sequence corresponding to SEQ ID NO:3. The RNA-dependent RNA polymerase has an amino acid sequence as given in SEQ ID NO:4 and a molecular weight from about 59 to about 63 kDa, preferably about 61 kDa.

Another such DNA molecule comprises an open reading frame which codes for a grapevine leafroll virus hsp70-related protein or polypeptide and comprises the nucleotide sequence corresponding to SEQ ID NO:5. The hsp70-related protein has an amino acid sequence corresponding to SEQ ID NO:6 and a molecular weight from about 57 to about 61 kDa, preferably about 59 kDa.

Another such DNA molecule comprises an open reading frame which codes for a grapevine leafroll virus hsp90-related protein and comprises the nucleotide sequence corresponding to SEQ ID NO:7. The hsp90-related protein has an amino acid sequence corresponding to SEQ ID NO:8 and a molecular weight from about 53 to about 57 kDa, preferably about 55 kDa.

Another such DNA molecule comprises an open reading frame which codes for a grapevine leafroll virus coat protein or polypeptide. The DNA molecule comprises the nucleotide sequence corresponding to SEQ ID NO:9. The coat protein has an amino acid sequence as given in SEQ ID NO:10 and a

molecular weight from about 33 to about 43 kDa, preferably about 35 kDa.

Alternatively, the DNA molecule of the present invention can constitute an open reading frame which codes for a first undefined protein or polypeptide. This DNA molecule comprises the nucleotide sequence corresponding to SEQ ID NO:11. The first undefined protein or polypeptide has an amino acid sequence corresponding to that in SEQ ID NO:12 and a molecular weight from about 5 to about 7 kDa, preferably about 6 kDa.

Another such DNA molecule constitutes an open reading frame which codes for a second undefined grapevine leafroll virus protein or polypeptide and comprises the nucleotide sequence corresponding to SEQ ID NO:13. The second undefined protein or polypeptide has an amino acid sequence as given in SEQ ID NO:14 and a molecular weight from about 4 to about 6 kDa, preferably about 5 kDa.

Another such DNA molecule constitutes an open reading frame which codes for a grapevine leafroll virus coat protein repeat and comprises the nucleotide sequence corresponding to SEQ ID NO:15. The coat protein repeat has an amino acid sequence as given in SEQ ID NO:16 and a molecular weight from about 51 to about 55 kDa, preferably about 53 kDa.

Yet another such DNA molecule constitutes an open reading frame which codes for a third undefined grapevine leafroll virus protein or polypeptide and comprises the nucleotide sequence corresponding to SEQ ID NO:17. The third undefined protein or polypeptide has an amino acid sequence as given in SEQ ID NO:18 and a molecular weight from about 33 to about 39 kDa, preferably about 36 kDa.

Yet another DNA molecule which constitutes an open reading frame for a fourth undefined grapevine leafroll virus protein or polypeptide comprises the nucleotide seuqence corresponding to SEQ ID NO:19. The fourth undefined protein or polypeptide has an amino acid sequence as given in SEQ ID NO:20 and a molecular weight from about 17 to about 23 kDa, preferably about 20 kDa.

Yet another DNA molecule constitutes an open reading frame for a fifth undefined grapevine leafroll virus protein or polypeptide and comprises the nucleotide sequence corresponding to SEQ ID NO:21. The fifth undefine protein or polypeptide has an amino acid sequence as given in SEQ ID NO:22 and a molecular weight from about 17 to about 23 kDa, preferably about 20 kDa.

Yet another DNA molecule of the present invention consitutes an open reading frame for a sixth undefined protein or polypeptide and comprises the nucleotide sequence corresponding to SEQ ID NO:23. The sixth undefined protein or polypeptide has an amino acid sequence as given in SEQ ID NO:24 and a molecular weight from about 5 to about 9 kDa, preferably about 7 kDa.

Also encompassed by the present invention are fragments of the DNA molecules of the present invention. Suitable fragments capable of imparting grapevine leafroll resistance to grape plants are constructed by using appropriate restriction sites, revealed by inspection of the DNA molecule's sequence, to: (i) insert an interposon (Felley et al., Gene, 52:147-15 (1987), which is hereby incorporated by reference) such that truncated forms of the grapevine leafroll virus coat polypeptide or protein, that lack various amounts of the C-terminus, can be produced or (ii) delete various internal portions of the protein. Alternatively, the sequence can be used to amplify any portion of the coding region, such that it can be cloned into a vector supplying both transcription and translation start signals suitable for the desired host cell.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of nucleotides that have minimal influence on the properties, secondary structure and hydropathic nature of the encoded polypeptide. For example, the nucleotides encoding a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The nucleotide sequence may

also be altered so that the encoded polypeptide is conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The protein or polypeptide of the present invention is preferably produced in purified form (preferably, at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is isolated after lysing or sonication. After washing, the lysate pellet is resuspended in buffer containing Tris-HCl. During dialysis, a precipitate forms from this protein solution. The solution is centrifuged, and the pellet is washed and resuspended in the buffer containing Tris-HCl. Proteins are resolved by electrophoresis through an SDS 12% polyacrylamide gel.

The DNA molecule encoding the grapevine leafroll virus protein or polypeptide of the present invention can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the coding sequence into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences as well known in the art.

U.S. Patent No. 4,237,224 (Cohen and Boyer), hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced, e.g., by transformation, and replicated in unicellular cultures including procaryotes and eucaryotic cells grown in culture.

Recombinant genes may also be introduced into virus vectors, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC184, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see Stratagene Cloning Systems Catalog (1993) from Stratagene, La Jolla, CA, hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see Studier et. al., Gene Expression Technology, vol. 185 (1990), hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1982), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria or transformed via particle bombardment (i.e. biolistics). The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable and well known transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., transcription and messenger RNA (mRNA) translation). Transcription of DNA is dependent upon the presence of a promotor, a DNA sequence that directs the binding of RNA polymerase and thereby promotes

mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals, including enhancer-like sequences and inducible regulatory sequences, may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are usually not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals, which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site (Shine-Dalgarno (SD) sequence) on the mRNA. This sequence is a short nucleotide sequence that is located before the start codon, usually AUG, which encodes the N-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and promote binding of mRNA to ribosomes by duplexing with rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). It is generally desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the cloned gene of interest. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promoter, *lac* promotor, *trp* promotor, *recA* promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promotor or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used

to provide for transcription of the inserted coding sequence or other inserted nucleic acid.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operons, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., have different under regulatory mechanisms.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires a SD sequence about 7-9 bases 5' to the initiation codon (ATG) to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include, but are not limited to, the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli trp* E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecules encoding the various grapevine leafroll virus proteins or polypeptides, as described above, have been cloned into an expression system, they are ready to be incorporated in a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, yeast, mammalian cells, insect, plant, and the like.

The present invention also relates to RNA molecules which encode the various grapevine leafroll virus proteins or polypeptides described above. The transcripts can be synthesized using the host cells of the present invention by any of the conventional techniques. The mRNA can be translated either *in vitro* or *in vivo*. Cell-free systems typically include wheat-germ or reticulocyte extracts.

One aspect of the present invention involves using one or more of the above DNA molecules encoding the various proteins or polypeptides of a grapevine leafroll virus to transform grape plants in order to impart grapevine leafroll resistance to the plants. The mechanism by which resistance is imparted is not known. As hypothesized, the transformed plant can express the coat protein or polypeptide, and, when the transformed plant is inoculated by a grapevine leafroll virus, such as GLRaV-1, GLRaV-2, GLRav-3, GLRaV-4, GLRaV-5, or GLRaV-6, or combinations of these, the recombinantly expressed coat protein or polypeptide surrounds the virus, thereby preventing translation of the viral DNA.

In this aspect of the present invention the subject coding sequence incorporated in the plant can be constitutively expressed. Alternatively, expression can be regulated by a promoter which is activated by the presence of grapevine leafroll virus. Suitable promoters for these purposes include those from genes expressed in response to grapevine leafroll virus infiltration. Additional suitable plant promoters include those which induce downstream gene expression in response to wounding, in response to elicitors and in response to virus infection. In the alternative, a constitutive plant-expressible promoter can be used; it is preferred that the level of gene expression is sufficiently high to provide virus resistance but not so high as to be detrimental to the normal functioning of the cell and tissues in which it is expressed. Immediately upstream of the start of a coding sequence for a GLRaV-3 protein or polypeptide in an expression system (expression vector, for use in plants) it is desired that there be a Kozak consensus for translation

intiation (AAXXATGG, where X is any of the four nucleotides). Downstream of the end of the coding sequence for the virus protein or polypeptide, it is preferred that there be a polyadenylation signal functional in plants, such as that from the nopaline synthase gene, the octopine synthase gene or from the CaMV 35S gene. These sequences are well known in the plant biotechnology art.

The DNA coding sequences and/or molecules of the present invention can be utilized to impart grapevine leafroll resistance for a wide variety of grapevine plants. The DNA molecules are particularly well suited to imparting resistance to *Vitis* scion or rootstock cultivars. Scion cultivars which can be protected include those commonly referred to as Table or Raisin Grapes, such as Alden, Almeria, Anab-E-Shahi, Autumn Black, Beauty Seedless, Black Corinth, Black Damascus, Black Malvoisie, Black Prince, Blackrose, Bronx Seedless, Burgrave, Calmeria, Campbell Early, Canner, Cardinal, Catawba, Christmas, Concord, Dattier, Delight, Diamond, Dizmar, Duchess, Early Muscat, Emerald Seedless, Emperor, Exotic, Ferdinand de Lesseps, Fiesta, Flame seedless, Flame Tokay, Gasconade, Gold, Himrod, Hunisa, Hussiene, Isabella, Italia, July Muscat, Khandahar, Katta, Kourgane, Kishmishi, Loose Perlette, Malaga, Monukka, Muscat of Alexandria, Muscat Flame, Muscat Hamburg, New York Muscat, Niabell, Niagara, Olivette blanche, Ontario, Pierce, Queen, Red Malaga, Ribier, Rish Baba, Romulus, Ruby Seedless, Schuyler, Seneca, Suavis (IP 365), Thompson seedless, and Thomuscat. They also include those used in wine production, such as Aleatico, Alicante Bouschet, Aligote, Alvarelhao, Aramon, Baco blanc (22A), Burger, Cabernet franc, Cabernet, Sauvignon, Calzin, Carignane, Charbono, Chardonnay, Chasselas dore, Chenin blanc, Clairette blanche, Early Burgundy, Emerald Riesling, Feher Szagos, Fernao Pires, Flora, French Colombard, Fresia, Furmint, Gamay, Gewurztraminer, Grand noir, Gray Riesling, Green Hungarian, Green Veltliner, Grenache, Grillo, Helena, Inzolia, Lagrein, Lambrusco de Salamino, Malbec, Malvasia bianca, Mataro, Melon, Merlot, Meunier, Mission, Montua de

Pilas, Muscadelle du Bordelais, Muscat blanc, Muscat Ottonel, Muscat Saint-Vallier, Nebbiolo, Nebbiolo fino, Nebbiolo Lampia, Orange Muscat, Palomino, Pedro Ximenes, Petit Bouschet, Petite Sirah, Peverella, Pinot noir, Pinot Saint-George, Primitivo di Gioa, Red Veltliner, Refosco, Rkatsiteli, Royalty, Rubired, Ruby Cabernet, Saint-Emilion, Saint Macaire, Salvador, Sangiovese, Sauvignon blanc, Sauvignon gris, Sauvignon vert, Scarlet, Seibel 5279, Seibel 9110, Seibel 13053, Semillon, Servant, Shiraz, Souzao, Sultana Crimson, Sylvaner, Tannat, Teroldico, Tinta Madeira, Tinto cao, Touriga, Traminer, Trebbiano Toscano, Trousseau, Valdepenas, Viognier, Walschriesling, White Riesling, and Zinfandel. Rootstock cultivars which can be protected include Couderc 1202, Couderc 1613, Couderc 1616, Couderc 3309, Dog Ridge, Foex 33 EM, Freedom, Ganzin 1 (A x R #1), Harmony, Kober 5BB, LN33, Millardet & de Grasset 41B, Millardet & de Grasset 420A, Millardet & de Grasset 101-14, Oppenheim 4 (SO4), Paulsen 775, Paulsen 1045, Paulsen 1103, Richter 99, Richter 110, Riparia Gloire, Ruggeri 225, Saint-George, Salt Creek, Teleki 5A, Vitis rupestris Constantia, *Vitis californica*, and *Vitis girdiana*.

There is extensive similarity in the hsp70-related sequence regions of GLRaV-3 and other closteroviruses, such as tristeza virus. Consequently, the GLRaV-3 hsp70-related gene can also be used to produce transgenic cultivars other than grape, such as citrus, which are resistant to closteroviruses other than grapevine leafroll, including tristeza virus. These include cultivars of lemon, lime, orange, grapefruit, pineapple, tangerine, and the like, such as Joppa, Maltaise Ovale, Parson (Parson Brown), Pera, Pineapple, Queen, Shamouti, Valencia, Tenerife, Imperial Doblefina, Washington Sanguine, Moro, Sanguinello Moscato, Spanish Sanguinelli, Tarocco, Atwood, Australian, Bahia, Baiana, Cram, Dalmau, Eddy, Fisher, Frost Washington, Gillette, LengNavelina, Washington, Satsuma Mandarin, Dancy, Robinson, Ponkan, Duncan, Marsh, Pink Marsh, Ruby Red, Red Seedless, Smooth Seville, Orlando Tangelo, Eureka, Lisbon, Meyer Lemon, Rough Lemon,

Sour Orange, Persian Lime, West Indian Lime, Bearss, Sweet Lime, Troyer Citrange, and Citrus trifoliata.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers. It is particularly preferred to utilize embryos obtained from anther cultures.

The expression systems of the present invention can be used to transform virtually any plant tissue under suitable conditions. Tissue transformed in accordance with the present invention can be grown *in vitro* in a suitable medium to impart grapevine leafroll virus resistance. Transformed cells can be regenerated into whole plants such that the protein or polypeptide imparts resistance to grapevine leafroll virus in the intact transgenic plants. In either case, the plant cells transformed with the recombinant DNA expression system of the present invention are grown and express one of the above-described grapevine leafroll virus proteins or polypeptides and, thus, grapevine leafroll resistance.

One technique of transforming plants with the DNA molecules of the present invention is by contacting the tissue of such plants with an inoculum of a bacterium transformed with a vector comprising a gene of the present invention which imparts grapevine leafroll resistance. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C. Cells of the genus *Agrobacterium* can be used to transform plant cells and/or plant tissue. Suitable species include *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. *A. tumefaciens* (e.g., strains C58, LBA4404, or EHA105) is particularly useful due to its well-known ability to transform plants, plant tissue and plant cells.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. This technique is disclosed in U.S. Patent Nos. 4,945,050,

5,036,006, and 5,100,792, all to Sanford et al., and in Emerschad et al., Plant Cell Reports, 14:6-12 (1995)), all hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Once grape plant tissue is transformed in accordance with the present invention, it is regenerated to form a transgenic grape plant. Generally, regeneration is accomplished by culturing transformed tissue on medium containing the appropriate growth regulators and nutrients to allow for the initiation of shoot meristems. Appropriate antibiotics are added to the regeneration medium to inhibit the growth of *Agrobacterium* and to select for the development of transformed cells. Following shoot initiation, shoots are allowed to develop in tissue culture and are screened for marker gene activity.

The DNA molecules of the present invention can be transcribed into mRNA, which, although encoding a grapevine leafroll virus protein or polypeptide, is not translated to the corresponding protein. This is known as RNA-mediated resistance. When a *Vitis* scion or rootstock cultivar is transformed with such a DNA molecule, the DNA molecule can be transcribed under conditions effective to maintain the mRNA in the plant cell at low level density readings. Density readings of between 15 and 50 using a Hewlet ScanJet and Image Analysis Program are preferred.

The grapevine leafroll virus protein or polypeptide can also be used to raise antibodies or binding portions thereof or probes. The antibodies can be monoclonal or polyclonal.

Monoclonal antibody production may be effected by techniques which are well-known in the art. Basically, the process involves first obtaining immune cells (lymphocytes) from the spleen of a mammal (e.g., mouse) which has been previously immunized with the antigen of interest either *in vivo* or *in vitro*. The antibody-secreting lymphocytes are then fused with (mouse) myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The resulting fused cells, or hybridomas, are cultured, and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, and grown either *in vivo* or *in vitro* to produce large quantities of antibody. A description of the theoretical basis and practical methodology of fusing such cells is set forth in Kohler and Milstein, Nature, 256:495 (1975), incorporated by reference.

Mammalian lymphocytes are immunized by *in vivo* immunization of the animal (e.g., a mouse) with the protein or polypeptide of the present invention. Such immunizations are repeated as necessary at intervals of up to several weeks to obtain a sufficient titer of antibodies. Following the last antigen boost, the animals are sacrificed and spleen cells removed.

Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, for example, by using polyethylene glycol (PEG) or other fusing agents. (See Milstein and Kohler, Eur. J. Immunol., 6:511 (1976), incorporated by reference.) This immortal cell line, preferably murine, but may also be derived from cells of other mammalian species, including but not limited to rats and humans, is selected to be deficient in enzymes necessary for the utilization of certain nutrients, capable of rapid growth,

and having good fusion capability. Many such cell lines are known to those skilled in the art, and others are regularly described.

Procedures for raising polyclonal antibodies are also well known. Typically, such antibodies can be raised by administering the protein or polypeptide of the present invention subcutaneously to New Zealand white rabbits which have first been bled to obtain pre-immune serum. The antigens can be injected at a total volume of 100 μl per site at six different sites. Each injected material will contain synthetic surfactant adjuvant pluronic polyols, or pulverized acrylamide gel containing the protein or polypeptide after SDS-polyacrylamide gel electrophoresis. The rabbits are then bled two weeks after the first injection and periodically boosted with the same antigen three times every six weeks. A sample of serum is then collected 10 days after each boost. Polyclonal antibodies are then recovered from the serum by affinity chromatography using the corresponding antigen to capture the antibody. Ultimately, the rabbits are euthanized with pentobarbital 150 mg/Kg IV. This and other procedures for raising polyclonal antibodies are disclosed in Harlow et. al., editors, Antibodies: A Laboratory Manual (1988), which is hereby incorporated by reference.

In addition to utilizing whole antibodies, binding portions of such antibodies can be used. Such binding portions include Fab fragments, $F(ab')_2$ fragments, and Fv fragments. These antibody fragments can be made by conventional procedures, such as proteolytic fragmentation procedures, as described in Goding, Monoclonal Antibodies: Principles and Practice, New York:Academic Press, pp. 98-118 (1983), hereby incorporated by reference.

The present invention also relates to probes found either in nature or prepared synthetically by recombinant DNA procedures or other biological procedures. Nucleic acid probes can also be synthesized by manual chemical synthesis (see, e.g., Beaucage and Caruthers (1981) Tetra. Lett. 22:1859-1862; MAtteuci et al. (1981) J. Am. chem. Soc.

103:3185) or by automated chemical synthesis using commercially available equipment (e.g., Applied Biosystems, Foster City, CA). Suitable probes are molecules which bind to grapevine leafroll viral antigens identified by the monoclonal antibodies of the present invention. Such probes can be, for example, proteins, peptides, lectins, or nucleic acid probes.

The antibodies or binding portions thereof or probes can be administered to grapevine leafroll virus infected scion cultivars or rootstock cultivars. Alternatively, at least the binding portions of these antibodies can be sequenced, and the encoding DNA synthesized. The encoding DNA molecule can be used to transform plants together with a promoter which causes expression of the encoded antibody or binding portion thereof when the plant is infected by grapevine leafroll virus. In either case, the antibody or binding portion thereof or probe will bind to the virus and help prevent the usual leafroll response.

Antibodies raised against the proteins or polypeptides of the present invention or binding portions of these antibodies can be utilized in a method for detection of grapevine leafroll virus in a sample of tissue, such as tissue from a grape scion or rootstock. Antibodies or binding portions thereof suitable for use in the detection method include those raised against a helicase, an RNA-dependent RNA polymerase, an hsp70-related, an hsp90-related, or a coat protein or polypeptide in accordance with the present invention. Any reaction of the sample with the antibody is detected using a reporter or other assay system which indicates the presence of grapevine leafroll virus in the sample. A variety of assay systems can be employed, such as enzyme-linked immunosorbent assays, radioimmunoassays, gel diffusion precipitin reaction assays, immunodiffusion assays, agglutination assays, fluorescent immunoassays, protein A immunoassays, or immunoelectrophoresis assays.

Alternatively, grapevine leafroll virus can be detected in such a sample using a nucleotide sequence of the DNA molecule, or a fragment thereof, encoding for a protein or

polypeptide (or a portion thereof) of the present invention. The nucleotide sequence is provided as a probe in a nucleic acid hybridization assay or a specific gene amplification detection procedure (e.g., using a polymerase chain reaction procedure). Any reaction with the probe is detected so that the presence of grapevine leafroll virus in the sample is indicated.

The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope. References cited in the Examples are incorporated by reference herein.

EXAMPLES

Example 1 - Materials and Methods

Virus purification and dsRNA isolation. The NY1 isolate, also referred to as isolate GLRaV 109 by Golino, Amer. J. Enol. Vitic, 43:200-205 (1992), a member of GLRaV-3 (Hu et al., J. Phytopathol. (Berl.), 128:1-14 (1990)); Zee et al., Phytopathology, 77:1427-1434 (1987)) was used throughout this work. Leafroll-diseased canes and mature leaves were collected from a vineyard in central New York State, and kept at -20°C until used. GLRaV-3 virus particles were purified according to the method described by Zee (1987), and modified later by Hu (1990). After two cycles of Cs_2SO_4 gradient purification, virus particles were observed from virus-enriched fractions by negative staining on an electron microscope.

The dsRNA was extracted from scraped bark/phloem tissue of canes as described in Hu (1990). Briefly, total nucleic acid was extracted with phenol/chloroform; dsRNA was absorbed on a CF-11 cellulose column under 17% ethanol and eluted without ethanol. After two cycles of ethanol precipitation, dsRNA was analyzed by electrophoresis on a 6% polyacrylamide or 1% agarose gel. A high *Mr* dsRNA (~16 kb) along with several smaller *Mr* dsRNAs was consistently identified in leafroll diseased but not in healthy samples (Hu (1990). The

16 kb dsRNA, which was presumably a replicative form of the virus, was purified further following separation on a low melting temperature-agarose gel (Sambrook et al., Molecular Cloning. A Laboratory Manual. 2nd Ed., Cold Spring Harbor Laboratory Press (1989). The double-stranded nature of the dsRNA was confirmed by its resistance to DNase and RNase in high salt and sensitivity to RNase in water (Hu (1990).

cDNA synthesis and molecular cloning. Complementary DNA (cDNA) was prepared by the procedure of Gubler et al., Gene, 25:263 (1983), and modified for dsRNA by Jelkmann et al., Phytopathology, 79:1250-1253 (1989). Briefly, following denaturation of about 2 μg of dsRNA in 20 mM methylmercuric hydroxide (MeHg) for 10 min, the first-strand cDNA was synthesized by avian myeloblastosis virus (AMV)-reverse transcriptase using random primers (Boehringer Mannheim, Indianapolis, IN). The second-strand cDNA was synthesized with DNA polymerase I while RNA templates were treated with RNase H. The cDNA was size-fractionated on a CL-4B Sepharose column and peak fractions, which contained larger molecular weight cDNA, were pooled and used for cloning. Complementary DNA ends were blunted with T4 DNA polymerase, and EcoRI adapters were ligated onto a portion of the blunt-ended cDNA. After treatment with T4 polynucleotide kinase and removal of unligated adapters by spin column chromatography, the cDNA was ligated with lambda ZAPII/EcoRI prepared arms (Stratagene, La Jolla, CA). These recombinant DNAs were packaged *in vitro* with GIGAPACK II GOLD™ packaging extract according to the manufacturer's instruction (Stratagene). The packaged phage particles were used to infect bacteria, *E. coli* XL1-blue cells.

Screening the cDNA library. To select GLRaV-3 dsRNA specific cDNA clones, probes were prepared from UNI-AMP™ (Clontech, Palo Alto, CA) PCR-amplified cDNA. PCR-amplified GLRaV-3 cDNA was labeled with ^{32}P [a-dATP] by Klenow fragment of *E. coli* DNA polymerase I with random primers and used as a probe for screening the library (Feinberg et al., Analytic Biochem., 132:6-13 (1983)). Library screening was carried out

by transferring plaques grown overnight onto GENESCREEN PLUS™ filters, following the manufacturer's instructions for denaturation, prehybridization, and hybridization (Dupont, Boston, MA). After washing, an autoradiograph was developed after exposing Kodak X-OMAT film to the washed filters overnight at -80°C. Bacteriophage recombinants were converted into plasmids (*in vivo* excision) following the manufacturer's instruction (Stratagene).

Identification of the coat protein gene was done by immunoscreening the cDNA library with GLRaV-3 specific polyclonal (Zee (1987)) and monoclonal (Hu (1990)), antibodies. Degenerate primer (5'GGNGGNGGNACNTTYGAYGTNTCN (SEQ. ID. No. 19), I=inosine, Y=T or C) generated from a conserved amino acid sequence in Motif C of the BYV HSP70 gene (p65) was used to select HSP70 positive clones. Further sequence extension was made possible by the clone walking strategy, which used sequences that flanked the sequence to probe the library for a clone that contained an insert extending farther in either 5' or 3' direction.

Northern blot hybridization. Inserts from selected clones were labeled with ^{32}P[a-dATP] by Klenow fragment of *E. coli* DNA polymerase I (Feinberg (1983)), and used as probes to test their specific reactions to dsRNAs isolated from leafroll infected tissues. Double-stranded RNA isolated from GLRaV-3 infected vines was separated by electrophoresis on a 1% agarose gel (nondenatured condition), denatured with 50 mM NaOH, 0.6 M NaCl for 30 min at room temperature, and neutralized with 1.5 M NaCl, 0.5 M Tris-HCl, pH 7.5 for another 30 min. Denatured dsRNA was sandwich-blotted onto a GENESCREEN PLUS™ membrane. Prehybridization and hybridization were carried out in a manner similar to that described above. The membrane was washed and exposed to Kodak X-OMAT film, and an autoradiograph was developed.

Identification of immunopositive clones. For immunoscreening, plates with plaques appearing after 8-12 h incubation at 37°C were overlaid with a 10 mM isopropyl-β-D-thio-galactopyranoside (IPTG)

impregnated Nylon filters (GENESCREEN PLUS™) and incubated for an additional 3-4 h. After blocking with 3% bovine serum albumin (BSA), the blotted filter was incubated in a 1:1000 dilution of alkaline phosphatase-conjugated GLRaV-3 polyclonal antibody for 3 h at 37°C. Positive signals (purple dots) were developed by incubation of washed filters in a freshly prepared nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) solution. To further confirm whether or not a true GLRaV-3 coat protein expression plaque was selected, a secondary immunoscreening was carried out by reinfection of bacterial XL1 Blue cells with an earlier selected plaque.

Western blot analysis. After secondary immunoscreening, GLRaV-3 antibody positive plaques were converted into plasmid, the pBluescript, by *in vivo* excision. Single colonies were picked up and cultured in LB medium with 100 μg/ml of ampicillin until mid-log growth. Fusion protein expression was induced by addition of 10 mM IPTG with an additional 3 h of incubation at 37°C. Bacteria was pelleted and denatured by boiling in protein denaturation buffer (Sambrook (1989)). An aliquot of 5 μl denatured sample was loaded and separated by electrophoresis on a 12% SDS-polyacrylamide gel along with a prestained protein molecular weight marker (Bio-Rad, Hercules, CA). The separated proteins were transferred onto an Immobulon membrane (Millipore) with an electroblotting apparatus (Bio-Rad). After blocking with 3% BSA, the transferred membrane was incubated with 1:1,000 dilution of either GLRaV-3 polyclonal or monoclonal antibody/alkaline phosphatase conjugate. A positive signal was developed after incubation of the washed membrane in NBT and BCIP.

PCR analysis. To analyze a cloned insert, an aliquot of a bacterial culture was used directly in PCR amplification with common vector primers (SK and KS). PCR-amplified product was analyzed by electrophoresis on an agarose gel.

Nucleotide sequencing and computer sequence analysis. Plasmid DNA, purified by either a CsCl method (Sambrook (1989)) or a modified mini alkaline-lysis/PEG precipitation

procedure (Applied Biosystems' Instruction), was sequenced either with Sequenase version 2 kit following the manufacturer's instruction (US Biochemical, Cleveland, Ohio) or with Taq DYEDEOXY™ terminator cycle sequencing kit (Applied Biosystems, Inc.). Automated sequencing was conducted on an ABI373 automated sequencer.

Nucleotide sequences were analyzed using a Genetics Computer Group (GCG) sequence analysis software package (Madison, WI). Sequence fragments were assembled using Newgelstart to initiate the GCG fragment assembly system and to support automated fragment assembly in GCG Version 7.2.

Computer-assisted analysis of phylogenetic relationships. Amino acid sequences were either obtained from database Swiss-Prot or translated from nucleotide sequences obtained from GenBank. A phylogenetic tree depicting a predicted relationship in the evolution of the GLRaV-3 coat protein sequence with those of other filamentous plant viruses was generated using the Clustal Method of the DNASTAR's MegAlign program (Madison, WI). With the Clustal method, a preliminary phylogeny is derived from the distances between pairs of input sequences and the application of the UPGMA algorithm (Sneath et al., Numerical Taxonomy - The Principles and Practice of Numerical Taxonomy, Freeman Press (1973)), which guides the alignment of ancestral sequences. The final phylogeny is produced by applying the neighborhood joining method of Saitou et al., Mol. Biol. Evol., 4:406-425 (1987), to the distance and alignment data.

Nucleotide sequence and primer selection. The sequence fragment (Table 16) selected for PCR has now been identified to be from nucleotides 9364 to 10,011 of the incomplete GLRaV-3 genome (Table 4). This sequence region encodes a short peptide which shares sequence similarity to HSP90 homologues of other closteroviruses (Figure 1). Selected primers and their designations are shown in Table 16, which shows the nucleotide and amino acid sequences of a PCR amplified fragment of the GLRaV-3 genome. The external and internal

primers used for PCR are underlined and their orientations are indicated by arrows.

Sample preparation. These include 1) dsRNA, 2) purified virus, 3) partially purified virus, 4) proteinase K treated crude extract, and 5) immuno-capture preparation.

Isolation of dsRNA from leafroll infected grapevine tissues followed the procedure developed by Hu (1990).

Virus purification was effected by the following procedure. An aliquot of 500 µl GLRaV-3-enriched fractions after two cycles of Cs_2SO_4 gradient was diluted with two volumes of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and incubated on ice for 5 min. The reaction was then adjusted to a final concentration of 200 mM NaAc, pH 5.0, 0.5% SDS, and 200 µg/ml proteinase K and incubated at 37°C for 3 h. Viral RNA was extracted with phenol and chloroform, ethanol-precipitated, and resuspended in 50 µl of diethyl pyrocarbonate (DEPC)-treated H_2O. For each 100 µl PCR reaction mixture, 1 µl of purified viral RNA was used as template.

Partially purified virus was prepared according to the virus purification procedure described in Hu (1990), but only to the high speed centrifugation (27,000 rpm, 2 h) step without further Cs_2SO_4 gradient centrifugation. The pellet was resuspended in TE buffer and subjected to proteinase K treatment as described above. Viral RNA was extracted with phenol/chloroform and precipitated using ethanol. From 10 g of starting material, the pellet was resuspended in 200 µl of DEPC treated H_2O. A 1 µl aliquot of extracted RNA or its 10-fold dilution series (up to 10^{-5}) was used for reverse transcription-PCR (RT-PCR).

Crude extract was treated with Proteinase K as follows. Liquid nitrogen powdered grapevine bark/phloem tissue (100 mg) was macerated in 1 ml of virus extraction buffer (0.5 M Tris-HCl, pH 9.0, 0.01 M $MgSO_4$, 4% water insoluble polyvinyl pyrrolidone (PVP40), 0.5% bentonite, 0.2% 2-mercaptoethanol, and 5% Triton X-100) (Zee (1987)). After a brief centrifugation (5,000 rpm, 2 min), 500 µl of supernatant was transferred into a new tube, adjusted to 100 µg/ml proteinase

K, and incubated for 1 h at 55°C (Kawasaki, "Sample Preparation from Blood, Cells, and Other Fluids," in Innis et al., eds, PCR Protocols: A Guide to Methods and Applications, Academic Press, Inc. (1990)). Following incubation, the preparation was boiled for 10 min to inactivate proteinase K and to denature the viral RNA. The upper clear phase was transferred into a new tube after a brief centrifugation. The viral RNA was precipitated with ethanol and resuspended in 100 µl of DEPC-treated H_2O. An aliquot of 1 µl proteinase K-treated crude extract or its 10-fold dilution series (up to 10^{-6}) was used.

The immuno-capture procedure was adapted from the method described by Wetzel et al., J. Virol. Meth. 39:27-37 (1992)). A 0.5 ml thin wall PCR tube was coated directly with 100 µl of 10 µg/ml purified gamma-globulin from GLRaV-3 antiserum (Zee (1987)) in ELISA coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6, and 0.02% NaN_3) and incubated for 4 h at 30°C. After washing 3 times with PBS-Tween-20, the antibody coated tube was loaded with 100 µl of crude extract (1:10 or its 10-fold dilution series, up to 10^{-6}) prepared in ELISA extraction buffer (50 mM sodium citrate, pH 8.3, 20 mM sodium diethyldithiocarbonate (DIECA), 2% PVP 40K) and incubated at 30°C for 4 h. After washing, a 25 µl aliquot of transfer buffer (10 mM Tris, pH 8.0, 1% Triton X-100) was added to the tube and vortexed thoroughly to release viral RNA.

RT-PCR. Initially, reverse transcription (RT) and polymerase chain reaction (PCR) were performed in two separate reactions. An aliquot of 20 µl of reverse transcription reaction mixture was prepared to contain 2 µl of 10X PCR buffer (Promega, Madison, WI) (10 mM Tris-HCl, pH 8.3, 500 mM KCl, and 0.01% gelatin), 50 mM $MgCl_2$, 2 µl of 10 mM dNTP, 150 ng of 5' and 3' primers, 16 units of RNasin, 25 units of avian myeloblastosis virus (AMV) reverse transcriptase, and 1 µl of a denatured sample preparation. The reverse transcription reaction was carried out at 37°C for 30 min. After denaturation by heating at 95°C for 5 min, an aliquot of PCR reaction mixture was added. This PCR reaction mixture (80 µl)

contained 8 μl of 10X PCR buffer (Promega), 150 mM $MgCl_2$, 250 ng of each 5' and 3' primer, 1 μl of 10 mM dNTP, and 2.5 units of Taq DNA polymerase. The thermal cycling program was set as follows: a precycle at 92°C for 3 min; followed by 35 cycles of denaturation at 92°C, 1 min; annealing at 50°C, 1 min; and extension at 72°C, 2.5 min. The final extension cycle was set at 72°C for 5 min.

Because reverse transcriptase functions in the PCR buffer system, RT and PCR can be combined (RT-PCR) in a single reaction (Ali et al., Biotechniques, 15:40-42 (1993); Goblet et al., Nucleic Acids Research, 17:2144 (1989)). The RT-PCR reaction mixture of 100 μl contains 10 μl of 10X PCR amplification buffer (Promega), 200 mM $MgCl_2$, 250 ng each of primers, 3 μl of 10 mM dNTPs, 40 units of RNasin, 25 units of AMV or moloney-murine leukemia virus (M-MLV) reverse transcriptase, 2.5 units of Taq DNA polymerase, and 1 μl of denatured sample preparation. The thermal cycling program was set as follows: one cycle of cDNA synthesis step at 37°C for 30 min, immediately followed by PCR cycling as described above.

Nested PCR. Inconsistent results obtained from a single round of PCR amplification prompted an investigation into the feasibility of Nested PCR. Initial PCR amplification was performed with an external primer set (93-110 & 92-98) (Table 15). A PCR product of 648 bp was consistently observed from dsRNA as template, but the expected PCR product was not consistently observed in samples prepared from proteinase K-treated crude extract or immuno-capture sample preparation. Consequently, additional PCR amplification with an internal primer set (93-25 & 93-40) was carried out by adding 5 μl of the first external primer-amplified PCR product into a freshly prepared 100 μl PCR reaction mixture. The PCR cycling parameters were as described above.

Example 2 - Virus Purification and dsRNA Isolation.

GLRaV-3 virus particles were purified directly from field collected samples of infected grapevines. Attempts to use

genomic RNA for cDNA cloning failed due to low yield of virus particles with only partial purity. However, virus particles were shown to be decorated by GLRaV-3 antibody using electron microscopy. The estimated coat protein molecular weight of 41K agreed with an earlier study (Hu (1990). Because of low yield in virus purification, dsRNA isolation was further pursued. Based on the assumption that high *Mr* dsRNA (16 kb) is the replicative form of the GLRaV-3 genomic RNA, this high *Mr* dsRNA was separated from other smaller ones by electrophoresis (Figure 2), purified from a low melting temperature agarose gel, and used for cDNA synthesis.

Example 3 - cDNA Synthesis, Molecular Cloning, and Analysis of cDNA Clones.

First-strand cDNA was synthesized with AMV reverse transcriptase using purified 16 kb dsRNA which had been denatured with 10 mM MeHg as template. Only random primers were used to prime the denatured dsRNA because several other closteroviruses (BYV, CTV, and LIYV) have been shown to have no polyadenylated tail on the 3' end (Agranovsky et al., J. Gen. Virol., 72:15-24 (1991)); Agranovsky et al., Virology, 198:311-324 (1994); Karasev et al., Virology, 208:511-520 (1995); Klaassen et al., Virology, 208:99-110 (1995); Pappu et al., Virology, 199:35-46 (1994)). After second-strand cDNA synthesis, the cDNA was size-fractionated on a CL-4B Sepharose column, and peak fractions which contained larger molecular weight cDNA were pooled and used for cloning. An autoradiograph of this pooled cDNA revealed cDNA molecules up to 4 kb in size.

A lambda ZAPII library was prepared from cDNA that was synthesized with random primed, reverse transcription of GLRaV-3 specific dsRNA. Initially, white/blue color selection in IPTG/X-gal containing plates was used to estimate the ratio of recombination. There were 15.7% white plaques, and an estimated 7 X 10^4 GLRaV-3 specific recombinants in this cDNA library. The library was screened with probes prepared from UNI-AMP™ PCR-amplified GLRaV-3 cDNA. More than 300 clones

with inserts of up to 3 kb were selected after screening the cDNA library with probe prepared from UNI-AMP™ PCR-amplified GLRaV-3 cDNA. In Northern blot hybridization, a probe prepared from a clone insert, pC4, reacted strongly to the 16 kb dsRNA as well as to several other smaller *Mr* dsRNAs. Such a reaction was not observed with nucleic acids from healthy grape or with dsRNA of CTV (Figure 1).

Example 4 - Selection and Characterization of Immunopositive Clones

A total of 6 X 10^4 plaques were immunoscreened with GLRaV-3 specific polyclonal antibody. Three cDNA clones, designated pCP5, pCP8-4, and pCP10-1, produced proteins that reacted to the polyclonal antibody to GLRaV-3. GLRaV-3 antibody specificity of the clones was further confirmed by their reaction to GLRaV-3 monoclonal antibody. PCR analysis of cloned inserts showed that a similar size of PCR product (1.0-1.1 kb) was cloned in each immunopositive clone using primers corresponding to flanking vector seuqences (SK and KS). However, various sizes of antibody-reacting protein were produced from these clones, which suggested that individual clones were independent and contained different segments of the coat protein gene. The *Mr* of immunopositive fusion protein from clone pCP10-1 was estimated to be 50K in SDS-PAGE, which was greater than the native coat protein of 41K (compare lanes 1 to 4 in Figure 3). Immunopositive proteins produced in clone pCP5 (Figure 3, lane 2) and pCP8 (Figure 3, lane 3) were different in size and smaller than the native coat protein. Clone pCP5 produced a GLRaV-3 antibody-reacting protein of 29K. Clone pCP8-4, however, produced an antibody-reacted protein of 27K. Similar banding patterns were observed when either polyclonal (Figure 3A) or monoclonal (Figure 3B) antibodies were used in Western blots. These results indicated that these cDNA clones contained coding sequences for the GLRaV-3 coat protein gene.

Example 5 - Nucleotide Sequencing and Identification of the Coat Protein Gene

Both strands of the three immunopositive clones were sequenced at least twice. A multiple sequence alignment of these three clones overlapped and contained an incomplete ORF lacking the 3' terminal sequence region. The complete sequence of this ORF was obtained by sequencing an additional clone, pA6-8, which was selected using the clone walking strategy. The complete ORF potentially encoded a protein of 313 amino acids with a calculated *Mr* of 34,866 (p35) (Figure 4 and Tables 2-3). Table 2 shows the nucleotide and amino acid sequences of the coat protein gene of grapevine leafroll associated closterovirus-3, isolate NY1. Nucleotide sequencing was conducted by the procedure described in Example 1. The translated amino acid sequence is shown below the nucleotide sequence. Table 3 compares the alignment of the coat protein of GLRaV-3 with respect to BYV, CTV, and LIYV. Consensus amino acid residues are shown. Uppercase letters indicate identical amino acids, and lowercase letters indicate at least three identical or functionally similar amino acids. The three conserved amino acid residues (S, R, and D) identified in all filamentous plant virus coat proteins are in bold (Dolja et al., Virology, 184:79-86 (1991)).

Because this ORF was derived from three independent clones after screening with GLRaV-3 coat protein specific antibody, it was identified as the coat protein gene of GLRaV-3. A multiple amino acid sequence alignment of p35 with the coat proteins of other closteroviruses, including BYV, CTV, and LIYV, is presented in Table 3. The typical consensus amino acid residues (S, R, and D) of the coat proteins of the filamentous plant viruses (Dolja et al., Virology, 184:79-86 (1991)), which may be involved in salt bridge formation and the proper folding of the most conserved core region (Boyko et al., Proc. Natl. Acad. Sci. USA, 89:9156-9160 (1992)), were also preserved in the p35. Phylogenetic analysis of the GLRaV-3 coat protein amino acid sequence with respect to the other filamentous plant viruses placed GLRaV-3 into a separate

but closely related branch of the closterovirus (Figure 5). Direct sequence comparison of GLRaV-3 coat protein with respect to other closterovirus coat proteins or their diverged copies by the GCG Pileup program demonstrated that at the nucleotide level, GLRaV-3 had its highest homology to BYV (41.5%) and CTV (40.3%). At the amino acid level, however, the highest percentage similarity were to the diverged copies of coat protein, with 23.5% identity (46.5% similarity) to CTV p26 and 22.6% (44.3% similarity) to BYV p24.

Example 6 - Identification of a Coat Protein Translation Initiation Site

Various sizes of GLRaV-3 specific antibody-reactive proteins were produced by three immunopositive clones in *E. coli* (Figure 3). Sequences of these clones overlapped and represented a common ORF that was identified as the coat protein gene (Figure 4). In searching for possible translation regulatory elements, sequence analysis beyond the coat protein coding region revealed a purine rich sequence, -uGAGuGAAcgcgAUG- (SEQ ID NO:26), which was similar to the Shine-Dalgarno sequence (uppercase letters) (Shine et al., Proc. Natl. Acad. Sci. USA, 71:1342-1346 (1974),), upstream from the coat protein initiation site (AUG). This purine rich sequence can serve as an alternative ribosome entry site for the translation of the GLRaV-3 coat protein gene in *E. coli*. If this first AUG in the ORF serves for coat protein translation, the ribosomal entry site must be located in this purine rich region because an in-frame translation stop codon (UGA) was only nine nucleotides upstream from the coat protein gene translation initiation site (AUG). Analysis of nucleotide sequence beyond the cloned insert into the vector sequence of clone pCP8-4 and pCP10-1 provided direct evidence that the fusion protein was made from the N-terminal portion of coat protein and C-terminal portion of β-galactosidase (16.5K). Further analysis of sequence around the selected AUG initiation codon of the coat protein gene revealed a consensus sequence

(-GnnAUGG-) that favored the expression of eucaryotic mRNAs (Kozak, Microbiological Reviews, 47:1-45 (1983); Kozak, Cell, 44:283-292 (1986)).

Nucleotide sequence analysis of three immunopositive clones revealed overlapping sequences and an ORF that covers about 96% of the estimated coat protein gene (Figure 4). The complete ORF was obtained after sequencing of an additional clone (pA6-8) that was selected by the clone walking strategy. Identification of this ORF as the coat protein gene was based upon its immunoreactivity to GLRaV-3 polyclonal and monoclonal antibodies, the presence of filamentous virus coat protein consensus amino acid residues (S, R, and D), and the identification of a potential translation initiation site. The calculated coat protein molecular weight (35K) is smaller than what was estimated on SDS-PAGE (41K). This discrepancy in molecular weight between computer-calculated and SDS-PAGE estimated falls in the expected range.

The estimated coat protein *Mr* of GLRaV-3 and another grape closterovirus-like designated GLRaV-1 are larger than the 22-28K coat protein range reported for other well characterized closteroviruses such as BYV, CTV, and LIYV (Agranovsky (1991); Bar-Joseph et al., "Closteroviruses," CMI/AAB, No. 260 (1982), Klaassen et al., J. Gen. Virol., 75:1525-1533 (1994); (Martelli et al., "Closterovirus, Classification and Nomenclature of Viruses, Fifth Report of the International Committee on Taxonomy of Viruses," in Archives of Virology Supplementum 2, Martelli et al., eds., New York: Springer-Verlag Wein, pp. 345-347 (1991); Sekiya et al., J. Gen. Virol., 72:1013-1020 (1991)). Hu (1990) suggested a possible coat protein dimer. The present sequence data, however, do not support this suggestion. First, the size of the coat protein is 35K, which is smaller than what would be expected of a coat protein dimer. Second, a multiple sequence alignment of N-terminal half and C-terminal half of GLRaV-3 coat protein with the coat proteins of other closteroviruses showed that the filamentous virus coat protein consensus amino acid residues (S, R, and D) are only present

in the C-terminal portion, but not in the N-terminal portion of the coat protein.

Example 7 - Primer Selection.

Primers were selected based on the nucleotide sequence of clone pC4 which had been shown to hybridize to GLRaV-3 dsRNAs on a Northern hybridization (Figure 1). The 648 bp sequence amplified by PCR was identified as nucleotides 9,364 to 10,011 of the incomplete GLRaV-3 genome (Table 4). This sequence fragment encodes a short peptide which shows some degree of amino acid sequence similarity to heat shock protein 90 (HSP90) homologues of other closteroviruses, BYV, CTV, and LIYV (Table 5). Two sets of primer sequences and their designations (external, 93-110 & 92-98, and internal, 93-25 & 93-40) are shown in Table 15. Effectiveness of synthesized primers to amplify the expected PCR product was first evaluated on its respective cDNA clone, pC4 (Figure 6, lane 11).

Example 8 - Development of a Simple and Effective PCR Sample Preparation.

Initially purified dsRNA was used in a RT-PCR reaction. The expected 219 bp PCR product was consistently observed with the internal set of primers (Figure 6, lane 10). To test whether or not these primers derived from GLRaV-3 specific dsRNA sequence is in fact the GLRaV-3 genome sequence, RNA extracted from a highly purified virus preparation was included in an assay. As expected, PCR products with similar size (219 bp) were observed in cloned plasmid DNA (pC4) (Figure 6, lane 11), dsRNA (Figure 6, lane 10) as well as purified viral RNA (Figure 6, lane 9). This PCR result was the first evidence that dsRNA isolated from leafroll-infected tissue was derived from the GLRaV-3 genome. However, PCR sample preparations from the purified virus procedure are too complicated to be used for leafroll diagnosis. Simplification sample preparations used viral RNA extracted from a partially purified virus preparation. This partially purified virus

preparation was again shown to be effective in RT-PCR (Figure 6). Sensitivity of RT-PCR was further evaluated with 10-fold serial dilution (up to 10^{-5}) of a sample. The expected PCR product of 219 bp in a partially purified virus preparation was observable up to the 10^{-3} dilution (Figure 6, lane 4). Although RT-PCR was shown again to work with partially purified virus preparations, this method of sample preparation was still too complicated to be used in a routine disease diagnosis. Over 10 attempts to directly use crude extract for RT-PCR were unsuccessful. Proteinase K-treated crude extract was by far the most simple and still effective pretreatment for RT-PCR. Therefore, the proteinase K-treated crude extract was used to evaluate RT-PCR for its ability to detect GLRaV-3.

Example 9 - RT-PCR

With proteinase K-treated crude extract prepared from scraped phloem tissue collected from a typical leafroll infected vine (Doolittle's vineyard, New York), a PCR product of 219 bp was readily observed. However, application of this sample preparation method to other field collected samples (USDA, PGRU, Geneva, NY) was disappointing. With different batches of sample preparations, a range of 3 to 10 out of 12 ELISA positive samples were shown to have the expected PCR products. To determine whether these inconsistent results were due to some kind of enzyme (reverse transcriptase or Taq DNA polymerase) inhibition present in the proteinase K-treated crude extract, increasing amounts of a sample were added into an aliquot of 100 μl PCR reaction mixture. PCR products of 219 bp were readily observed from samples of 0.1 μl (lane 1) and 1 μl (lane 2) but not from 10 μl. Presumably, sufficient amount of enzyme inhibitors was present in the 10 μl of this sample.

Example 10 - Immuno-capture RT-PCR

The immuno-capture method further simplified sample preparation by directly using crude extracts that were prepared in the standard ELISA extraction buffer. Immuno-

capture RT-PCR (IC RT-PCR) tests were initially performed with the internal primer set, and the expected PCR product of 219 bp was observed from a typical leafroll infected sample. However, this PCR method to test a range of field collected ELISA positive samples gave inconsistent results. In a PCR test performed with the external primer set, only five out of seven field collected ELISA positive samples were shown to amplify the expected PCR product (648 bp) (Figure 7A). Meanwhile, the expected PCR product was consistently observed in dsRNA (Figure 7A, lane 10), but such product was never observed in the healthy control (Figure 7A, lane 9). In this case, however, the expected PCR product was not observed in a sample prepared using proteinase K-treated crude extract (Figure 7A, lane 8).

Example 11 - Nested PCR

As described above, inconsistency of RT-PCR was experienced with samples prepared either by the proteinase K-treated or by the immuno-capture methods. If this PCR technique is to be used in disease diagnosis, a consistent and reproducible result is needed. Thus, the Nested PCR method was introduced. Although an expected PCR product of 648 bp from the first PCR amplification with the external primer set was not always observable (Figure 7A), in a Nested PCR amplification with the internal primer set, the expected 219 bp PCR product was consistently observed from all seven ELISA positive samples (Figure 7B). These products were observed in dsRNA (Figure 7B, lane 10) and in the proteinase K-treated crude extract (Figure 7B, lane 8) but not in a healthy control (Figure 7B, lane 9). To determine the sensitivity of Nested PCR with samples prepared either by proteinase K-treated or by immuno-capture methods, Nested PCR and ELISA were performed simultaneously with samples prepared from a 10-fold dilution series. The sensitivity of Nested PCR was shown to be 10^{-5} in proteinase K-treated crude extract (Figure 8A), and was more than 10^{-8} (the highest dilution point in this test) in an

immuno-capture preparation (Figure 8B). With similar sample preparations, sensitivity for ELISA was only 10^{-2}.

Example 12 - Validation of PCR with ELISA and indexing

To determine whether the PCR-based GLRaV-3 detection method described in this study has a practical application in grapevine leafroll disease diagnosis, a validation experiment with plants characterized thoroughly by ELISA and indexing is necessary. Several grapevines collected at USDA-PGRU at Geneva, New York, which have been well characterized by 3-year biological indexing and by ELISA were selected for validation tests. A perfect correlation was observed between ELISA positive and PCR positive samples, although there was some discrepancy over indexing which suggested that other types of closteroviruses may also be involved in the grapevine leafroll disease (Table 7).

PCR technology has been applied to detect viruses, viroids and phytoplasmas in the field of plant pathology (Levy et al., Journal of Virological Methods, 49:295-304 (1994)). Because of the presence of enzyme inhibitors (reverse transcriptase and/or Taq DNA polymerase) in many plant tissues, a lengthy and complicated procedure is usually required to prepare a sample for PCR. In studies of PCR detection of grapevine fanleaf virus, Rowhani et al., Phytopathology, 83:749-753 (1993), have already observed an enzyme inhibitory phenomenon. Substances including phenolic compounds and polysaccharides in grapevine tissues were suggested to be involved in enzyme inhibition.

One of the objectives in the present study was to develop a sound practical procedure for sample preparation to eliminate this inhibitory problem for PCR detection of GLRaV-3 in grapevine tissues. Although the expected PCR product was consistently observed from samples of dsRNA, purified virus and partial purified virus, proteinase K-treated crude extract and immuno-capture methods were the simplest and were still effective. Samples prepared with proteinase K-treated crude extract have an advantage over others in that hazardous

organic solvents, such as phenol and chloroform, are avoided. However, care must be taken in the sample concentration because the reaction can be inhibited by adding too much grapevine tissue. Minafra et al., J. Virol. Methods, 47:175-188 (1994), reported the successful PCR detection of grapevine virus A, grapevine virus B, and GLRaV-3 with crude saps prepared from infected grapevine tissues, this method of sample preparation was, however, not effective in the present study. The similar primers used by Minafra (1994), were, however, able to amplify the expected size of PCR products from dsRNA of the NY1 isolate of GLRaV-3.

Immuno-capture is another simple and efficient method of sample preparation (Wetzel (1992), which is hereby incorporated by reference). First, crude ELISA extracts can be used directly for RT-PCR. Second, it provides not only a definitive answer, but may also be an indication to a virus serotype. Third, with an immuno-capture step, virus particles are trapped by an antibody, and inhibitory substances may be washed away. Nested PCR with samples prepared by the immuno-capture method is 10^3 times more sensitive than with samples prepared by proteinase K-treated crude extract. However, this approach requires a virus specific antibody. For some newly discovered or hard to purify viruses, a virus specific antibody might not be available. There are at least six serologically distinctive closteroviruses associated with grapevine leafroll disease (Boscia (1995)).

Example 13 - Nucleotide Sequence and Open Reading Frames

A lambda ZAPII library was prepared from cDNA that was synthesized with random primed, reverse transcription of GLRaV-3 specific dsRNA. Initially, white/blue color selection in IPTG/X-gal containing plates was used to estimate the ratio of recombination. There were 15.7% white plaques, or an estimated 7 X 10^4 GLRaV-3 specific recombinants in this cDNA library. The library was screened with probes prepared from UNI-AMP™ PCR-amplified GLRaV-3 cDNA. More than 300 clones with inserts of up to 3 kb were selected after screening the

cDNA library with probe prepared from UNI-AMP™ PCR-amplified GLRaV-3 cDNA. In Northern blot hybridization, a probe prepared from the cloned insert of pC4, reacted strongly to the 16 kb dsRNA as well as to several other smaller *Mr* dsRNAs. No hybridization with nucleic acids from healthy grape or to dsRNA of CTV was observed (Figure 1).

Clone pB3-1 was selected and sequenced after screening the library with HSP70 degenerate primer (5'GGIGGIGGIACITTYGAYGTITCI (SEQ ID NO:25)). Other clones that were chosen for nucleotide sequencing were selected by the clone walking strategy. The nucleotide sequencing strategy employed was based on terminal sequencing of random selected clones assisted with GCG fragment assembly program to assemble and extend the sequence. The step-by-step primer extension method was used to sequence the internal region of a selected clone. A total of 54 clones were selected for sequencing. Among them, 16 clones were completely sequenced on both DNA strands (Figure 9).

A total of 15,227 nucleotides were sequenced; nine open reading frames (ORFs) were identified designated as ORFs 1a, 1b, and 2 to 8. The sequenced region was estimated to cover about 80% of the complete GLRaV-3 genome. Major genetic components, such as helicase (ORF 1a), RdRp (ORF 1b), HSP70 homologue (ORF 4), HSP90 homologue (ORF 5) and coat protein (ORF 6) were identified.

ORF 1a was an incomplete ORF from which the 5' terminal portion has yet to be cloned and sequenced. The sequenced region presented in Figure 10 and Table 4 represents approximately two-thirds of the expected ORF 1a , as compared to the ORF 1a from BYV, CTV, and LIYV. The partial ORF 1a was terminated by the UGA stop codon at positions 4165-4167; the respective product consisted of 1388 amino acid residues and had a deduced *Mr* of 148,603. Database searching indicated that the C-terminal portion of this protein shared significant similarity with the Superfamily 1 helicase of positive-strand RNA viruses. Comparison of the conserved domain region (291 amino acids) showed a 38.4% identity with an additional 19.7%

similarity between GLRaV-3 and BYV and a 32.4% identity with an additional 21.1% similarity between GLRaV-3 and LIYV (Table 6). Six helicase conserved motifs of Superfamily 1 helicase of positive-strand RNA viruses (Hodgman, Nature, 333:22-23 (Erratum 578) (1988); Koonin et al., Crit. Rev. Biochem. Molec. Biol., 28:375-430 (1993)) were also retained in GLRaV-3. Analysis of the predicted phylogenetic relationship in helicase domains between GLRaV-3 and the other positive-strand RNA viruses placed GLRaV-3 along with the other closteroviruses, including BYV, CTV, and LIYV, into the "tobamo" branch of the alphavirus-like supergroup (Figure 11, Table 5). Table 5 compares the amino acid sequence alignment of the helicase of GLRaV-3 with respect to BYV, CTV, and LIYV. Consensus amino acid residues are shown. Uppercase letters indicate identical amino acids, lowercase letters indicate at least three identical or functionally similar amino acids. Six conserved motifs (I to VI) that are conserved among the Superfamily 1 helicase (Koonin et al., Crit. Rev. in Biochem. Molec. Biol., 28:375-430 (1993)) of the positive-strand RNA viruses are overlined.

ORF 1b overlapped the last 113 nucleotides of ORF 1a and terminated at the UAG codon at positions 5780 to 5782. This ORF encodes a protein of 536 amino acid residues with a calculated *Mr* of 61,050 (Figures 10, Table 4). Database screening of this protein revealed significant similarity to the Supergroup 3 RdRp of the positive-strand RNA viruses. Sequence comparison of GLRaV-3 with BYV, LIYV, and CTV over a 313-amino acid sequence fragment revealed a striking amino acid sequence similarity among eight conserved motifs (Table 8). Consensus amino acid residues are shown. Uppercase letters indicate identical amino acids, and lowercase letters indicate at least three identical or functionally similar amino acids. The motifs (I to VIII) that are conserved among the Supergroup 3 RNA polymerase of positive-strand RNA viruses are overlined. The best alignment was with BYV, while the least alignment was with LIYV (Table 6). Analysis of predicted phylogenetic relationships of the RdRp domains of

the alphavirus-like supergroup viruses again placed GLRaV-3 into the tobamo branch along with other closteroviruses, BYV, CTV, BYSV, and LIYV (Figure 12).

Publications on BYV, CTV, and LIYV have proposed that ORF 1b is expressed via a +1 ribosomal frameshift (Agranovsky (1994); Dolja et al., Ann. Rev. Phytopathol., 32:261-285 (1994); Karasev (1995), and Klaassen (1995)). Direct nucleotide sequence comparison was performed within the ORF1a/1b overlap of GLRaV-3 with respect to BYV, CTV, or LIYV. An apparently significant similarity was observed only to LIYV (Table 9), and not to BYV or CTV. The so-called "slippery" GGGUUU sequence and the stem-and-loop structure that were proposed to be involved in the BYV frameshift was absent from the GLRaV-3 ORF1a/1b overlap. The predicted frameshift within the GLRaV-3 ORF 1a/1b overlap was selected based on an inspection of the C-terminal portion of the helicase alignment and the N-terminal portion of the RdRp alignment between GLRaV-3 and LIYV.

Table 9 compares the aligned GLRaV-3 and LIYV nucleotide sequences (presented as DNA) in the vicinity of the proposed frameshift, nt 4099-4165 in GLRaV-3 and nt 5649-5715 in LIYV. Identical nucleotides are uppercase. LIYV predicted +1 frameshift region (aAAG) and the corresponding GLRaV-3 (cACA) are bold and italic. The encoded C-terminus of HEL and N-terminus of RdRp are presented above (GLRaV-3) and below (LIYV) the nucleotide alignment. Repeat sequences are underlined.

The GLRaV-3 ORF 1a/1b frameshift was predicted to occur in the homologous region of the LIYV genome, and was also preceded by a repeat sequence (GCTT) (Figure 24). Unlike LIYV, this repeat sequence was not a tandem repeat and was separated by one nucleotide (T) in GLRaV-3.

The frameshift was predicted to occur at CACA (from His to Thr) in GLRaV-3 rather than slippery sequence AAAG in LIYV. However, additional experiments on *in vitro* expression of GLRaV-3 genomic RNA are needed in order to determine whether or not a large fusion protein is actually produced.

ORF 2 is predicted to encode a small peptide of 51 amino acids and a calculated *Mr* of 5,927. Database searching did not reveal any obvious protein matches within the existing Genbank (Release 84.0).

Intergenic regions of 220 bp between ORF 1b and ORF 2 and 1065 bp between ORF 2 and ORF 3 were identified. There is no counterpart in the BYV or LIYV genomes; instead, an ORF of 33K in CTV (Karasev et al., J. Gen. Virol., 75:1415-1422 (1994)) or 32K in LIYV (Klaassen (1995)) is observed over this similar region.

ORF 3 encodes a small peptide of 45 amino acids and a calculated *Mr* of 5,090 (p5K). Database searching revealed that it was most closely related to the small hydrophobic, transmembrane proteins of BYV (6.4K), CTV (6K), and LIYV (5K). Individual comparison (Table 3) showed that LIYV was its closest relative (45.8%) at the nucleotide level and BYV was the most homologous (30.4%) at the amino acid level.

Table 10 compares the aligned amino acid sequences the small hydrophobic transmembrane protein of GLRaV-3 p5K with those of BYV (p6K), CTV (p6K), and LIYV (p5K). Consensus amino acid residues are shown. Lowercase letters indicate at least three identical or functionally similar amino acids. The transmembrane domain that has been identified in several other closteroviruses, BYV, CTV, and LIYV (Karasev et al., Virology, 208:511-520 (1995)), is overlined.

ORF 4 encodes a protein of 549 amino acids and a calculated *Mr* of 59,113 (p59) (Figure 10, Table 4). Database screening revealed significant similarity to the HSP70 family, the p65 protein of BYV, the p65 protein of CTV, and the p62 protein of LIYV. A multiple amino acid sequence alignment of GLRaV-3 p59 with HSP70 analogs of other closteroviruses showed striking sequence similarity among eight conserved motifs (A-H). Functionally important motifs (A-C) that are characteristic of all proteins containing the ATPase domain of the HSP70 type (Bork et al., Proc. Natl. Acad. Sci. USA, 89:7290-7294 (1992)) were also preserved in GLRaV-3 p59, which suggested that this HSP70 chaperonin-like protein may also

possess ATPase activity on its N-terminal domain and protein-protein interaction on its C-terminal domain (Dolja (1994).

Table 11 presents the amino acid sequence alignment of the HSP70-related protein of GLRaV-3 (p59K) with those of BYV (p65K), CTV (p65K), and LIYV (p62K). The eight conserved motifs (A to H) of cellular HSP70 are overlined. Consensus amino acid residues are shown. Uppercase letters indicate identical amino acids, and lowercase letters indicate at least three identical or functionally similar amino acids.

Analysis of the predicted phylogenetic relationship of p59 of GLRaV-3 with HSP70-related proteins of other closteroviruses (BYV, CTV, and BYSV) and cellular HSP70s again placed the four closteroviruses together and the rest of the cellular HSP70s on the other branches (Figure 13). Although several closterovirus HSP70-related proteins are closely related to each other and distant from other cellular members of this family, inspection of the phylogenetic tree indicates that GLRaV-3 may be an ancestral closterovirus relatively early in evolution as predicted by Dolja (1994), because GLRaV-3 was placed in between closteroviruses and the other cellular HSP70 members.

ORF 5 encodes a protein of 483 amino acids with a calculated *Mr* of 54,852 (p55) (Figure 10, Table 4). Table 12 compares the alignment of the amino acid sequence deduced from the PCR fragment of GLRaV-3 with respective regions of HSP90 homologues of beet yellow virus (BYV) (p64), citrus tristeza virus (CTV) (p61), and lettuce infectious yellow virus (LIYV) (p59). Consensus amino acid residues are shown. Uppercase letters indicate identical amino acids, lowercase letters indicate at least three identical or functionally similar amino acids.

No significant sequence homology with other proteins was observed in the current database (GenBank, release 84.0). Direct comparison with other counterparts (p61 of CTV, p64 of BYV, and p59 of LIYV) of closteroviruses revealed some degree of amino acid sequence similarity, with 21.7% to BYV, 17.5% to CTV, and 16.7% to LIYV, respectively (Tables 6, 11, 12). Two

conserved regions of HSP90 previously described in BYV and CTV (Pappu (1994)) were identified in the p55 of GLRaV-3 (Table 13).

ORF 6 encodes a protein of 313 amino acids with a calculated *Mr* of 34,866 (p35) (Figure 10 and Table 4). The fact that this ORF was encoded by three overlapping GLRaV-3 immunpositive clones indicates that it contains the coat protein gene of GLRaV-3. Alignment of the product of ORF 6 (p35) with the coat protein sequences of BYV, CTV, and LIYV, is presented in Table 3. The typical consensus amino acid residues (S, R, and D) of the coat protein of the filamentous plant viruses (Dolja (1991)), which may be involved in salt bridge formation and the proper folding of the most conserved core region (Boyko (1992)), were also retained in the p35 (Table 3). Individual sequence comparison showed the highest similarity to CTV (20.5%) and BYV (20.3%), and the lowest similarity to LIYV (17.8%). Analysis of predicted phylogenetic relationships with other filamentous plant viruses tentatively placed GLRaV-3 into a separate, but a closely related branch of closteroviruses (Figure 5).

ORF 7 encodes a protein of 477 amino acids with a calculated *Mr* of 53,104 (p53) (Figure 10 and Table 4). Based on the presence of conserved seuqences, this protein is designated as grapevine leafroll virus coat protein repeat (p53).

ORF 8 encodes an undefined polypeptide of a calculated Mr of 21,248 (p21).

ORF 9 encodes an undefined protein of calculated Mr of 19,588 (p20).

ORF 10 encodes an undefined polypeptide with a calculated Mr of 19,653 (p20).

ORF 11 encodes an undefined protein of calculated Mr of 6963 (p7).

In the present study, many GLRaV-3 dsRNA specific cDNA clones were identified using a probe generated from UNI-AMP™ PCR-amplified cDNA. Using UNI-AMP™ adapters and primers (Clontech) in PCR has several advantages. First, it is not

necessary to know the nucleotide sequence of an amplified fragment. Second, cDNA can be amplified in sufficient amounts for specific probe preparation. In general, cDNA amplified by PCR using UNI-AMP™ primers and adapters could be used for cloning as well as a probe for screening of cDNA libraries. However, low abundance of the starting material and many cycles of PCR amplification often incorporate errors into the nucleotide sequence (Keohavong et al., Proc. Natl. Acad. Sci. USA, 86:9253-9257 (1989); Saiki et al., Science, 239:487-491 (1988)). In the present study, only UNI-AMP™ PCR amplified cDNA was used as a probe for screening. The cDNA library was generated by direct cloning of the cDNA that was synthesized by AMV reverse transcriptase. Therefore, the cDNA cloned inserts are believed to more accurately reflect the actual sequence of the dsRNA and the genomic RNA of GLRaV-3.

A total of 15,227 nucleotides or about 80% of the estimated 16 kb GLRaV-3 dsRNA was cloned and sequenced. Identification of this sequence fragment as the GLRaV-3 genome was based on its sequence alignment with the coat protein gene of GLRaV-3. This is the first direct evidence showing that high molecular weight dsRNA (~16 kb) isolated from GLRaV-3 infected vines is derived from GLRaV-3 genomic RNA. Based upon the nine ORFs identified, the genome organization of GLRaV-3 bears significant similarity to the other closteroviruses sequenced (BYV, CTV, and LIYV) (Figure 10).

Dolja (1994) tentatively divided the closterovirus genome into four modules. For GLRaV-3, the 5' accessory module including protease and vector transmission factor is yet to be identified. The core module, including key domains in RNA replication machinery (MET-HEL-RdRp) that is conserved throughout the alphavirus supergroup, has been revealed in parts of the HEL and RdRp domains. The MET domain has not yet been identified for GLRaV-3. The chaperon module, including three ORFs coding for the small transmembrane protein, the HSP70 homologue, and the distantly related HSP90 homologue, has been fully sequenced. The last module includes coat protein and its possible diverged copy and is also preserved

in GLRaV-3. Overall similarity of the genome organization of GLRaV-3 with other closteroviruses further support the inclusion of GLRaV-3 as a member of closteroviruses (Hu (1990) and Martelli (1991), which are hereby incorporated by reference). However, observation of a predicted ambisense gene on its 3' terminal region may separate GLRaV-3 from other closteroviruses. Further comparative sequence analysis (Table 3) as well as phylogenetic observation of GLRaV-3 with respect to other closteroviruses over the entire genome sequence region suggested that GLRaV-3 is most closely related to BYV, followed by CTV, and LIYV.

As suggested by others (Agranovsky (1994), Dolja (1994), Karasev (1995), and Klaassen (1995)), expression of ORF 1b in closteroviruses may be via a +1 ribosomal frameshift mechanism. In GLRaV-3, a potential translation frameshift of ORF 1b could make a fusion HEL-RdRp protein of over 1,926 amino acid residues with a capacity to encode a protein of more than 210K Comparative study of GLRaV-3 with respect to other closteroviruses over the ORF 1a/1b overlap revealed a significant sequence similarity to LIYV, but not to BYV or to CTV. The so-called slippery sequence (GGGUUU) and stem-loop and pseudoknot structures identified in BYV (Agranovsky (1994), which is hereby incorporated by reference) is not present in GLRaV-3. Thus, a frameshift mechanism that is similar to LIYV may be employed for GLRaV-3. However, protein analysis is necessary in order to determine the protein encoding capacities of these ORFs.

Differing from BYV, both CTV and LIYV have an extra ORF (ORF 2) in between RdRp (ORF 1b) and the small membrane protein (ORF 3) and potentially encoding a protein of 33K or 32K, respectively. However, in GLRaV-3, there is a much smaller ORF 2 (7K) followed by a long intergenic region of 1065 bp.

So far, among all plant viruses described, the HSP70 related gene is present only in the closteroviruses (Dolja (1994)). Identification of the GLRaV-3 HSP70 gene was based on an assumption that this gene should also be present in the

closterovirus associated with grapevine leafroll disease, specifically GLRaV-3. Thus, cDNA clones that reacted with HSP70-degenerated primers were identified for sequence analysis. The identification of subsequent clones for sequencing was based on the gene-walking methodology. However, identification of immunopositive clones enabled identification of the coat protein gene of GLRaV-3 and proved that the HSP70-containing sequence fragment is present in the GLRaV-3 RNA genome.

The 16 kb dsRNA used for cDNA synthesis was assumed to be a virus replicative form (Hu (1990). Selected clones have been shown by Northern hybridization to hybridize to the 16 kb dsRNA and several smaller RNAs (presumably subgenomic RNAs) (Figure 1). Second, three GLRaV-3 antibody-reacting clones were identified after immuno-screening of the protein expression library with both GLRaV-3 polyclonal (Zee (1987)) and monoclonal (Hu (1990)) antibodies. After nucleotide sequencing, these three antibody-reacting clones were shown to overlap one another and contain a common ORF which potentially encodes a protein with calculated *Mr* of 35K. This is consistent with the *Mr* estimated on SDS-PAGE (41K). Third, analysis of the partial genome sequence of GLRaV-3 suggested a close similarity in genome organization and gene sequences to the other closteroviruses (Dolja (1994)).

Information regarding the genome of GLRaV-3 provides a better understanding of this and related viruses and adds to the fundamental knowledge of closteroviruses. Present work on the nucleotide sequence and genome organization (about 80% of the estimated genome sequence) has provided direct evidence for a close relationship between GLRaV-3 and other closteroviruses. It has also enabled, for the first time, the evaluation of phylogenetic relationships of GLRaV-3 based on a wide range of genes and gene products (helicase, polymerase, HSP70 homologue, HSP90 homologue, and coat protein). Based upon major differences in genome format and organization between BYV, CTV, and LIYV, along with phylogenetic analysis, Dolja (1994)) proposed the establishment of the new family

Closteroviridae with three new genera of *Closterovirus* (BYV), *Citrivirus* (CTV), and *Biclovirus* (LIYV). This work on genome organization and phylogenetic analysis, along with evidence that this virus is transmitted by mealybugs (see hereinabove) indicates that a new genus under *Closteroviridae* family should be established. Thus, GLRaV-3 (the NY1 isolate) is proposed as the type representative of the new genus, *Gracilovirus* (*gra*-pevine *clo*-sterovirus). Further sequencing of other grapevine leafroll associated closteroviruses may add more members to this genus.

Another cDNA library of GLRaV-3 has been established recently from dsRNA of an Italian isolate of GLRaV-3 (Saldarelli et al., Plant Pathology (Oxford), 43:91-96 (1994), which is hereby incorporated by reference). Selected clones react specifically to GLRaV-3 dsRNA on a Northern blot; however, no direct evidence was provided to suggest that those clones were indeed from GLRaV-3 genomic RNA. Meanwhile, a small piece of sequence information from one of those cDNA clones was used to synthesize primers for the development of a PCR detection method (Minafra (1994), which is hereby incorporated by reference). Direct sequence comparison of these primer sequences to GLRaV-3 genome sequence obtained in the present study, showed that one of the primers (H229, 5'ATAAGCATTCGGGATGGACC (SEQ ID NO:27)) is located at nucleotides 5562-5581 and the other (C547, 5'ATTAACtTgACGGATGGCACGC (SEQ ID NO:28)) is in reverse direction and is the complement of nucleotides 5880-5901. Mismatching nucleotides between the primers and GLRaV-3 sequence are shown in lowercase letters. Sequence comparison over these short primer regions to GLRaV-3 (isolate NY1) genome sequence showed a 90-95% identity, which suggested that these two isolates belong to the same virus (GLRaV-3). Moreover, the primers prepared by Minafra (1994), which is hereby incorporated by reference, from the Italian isolate of GLRaV-3 produced an expected size of PCR product with templates prepared from the NY1 isolate of GLRaV-3.

The remainder of the GLRaV-3 genome can be readily sequenced using the methods described herein and/or techniques well known to the art.

Example 14 - Identification and Characrerization of the 43 K ORF

The complete nucleotide sequence of the GLRaV-3 HSP90-related gene is given in Table 4. Initial sequencing work indicated that an open reading frame (ORF) encoding for a protein with a calculated *Mr* of 43K (Table 14) was downstream of the HSP70-related gene. This gene was selected for engineering because the size of its encoded product is similar to the GLRaV-3 coat protein gene. However, after sequence analysis, this incomplete ORF was located in the 3' terminal region of the HSP90-related gene. It is referred to herein as the incomplete GLRaV-3 HSP90 gene or as the 43K ORF.

Example 15 - Custom-PCR Engineering the Incomplete GLRaV-3 HSP90 Gene for Expression in Plant Tissues

Two custom synthesized oligonucleotide primers, 5' primer (93-224, tacttatctagaaccATGGAAGCGAGTCGACGACTA (SEQ ID NO:29)) and 3' complementary primer (93-225, tcttgaggatccatggAGAAACATCGTCGCATACTA (SEQ ID NO:30)) that flank the 43K ORF were designed to amplify the incomplete HSP90 gene fragment by polymerase chain reaction. Addition of a restriction enzyme NcoI site in the primer facilitates cloning and protein expression (Table 15) (Slightom, Gene, 100:251-255 (1991)). Using these primers, a product of the proper size (1.2 kb) was amplified by RT-PCR using GLRaV-3 double-stranded RNA (dsRNA) as template.

Table 14 shows the nucleotide sequence fragment containing the 43 kDa open reading frame that used to engineer the plant expression cassette, pBI525GLRaV-3hsp90. This fragment (nucleotides 9404 to 10,503 of the partial GLRaV-3 genome sequence, Table 4) is located in the 3' portion of GLRaV-3 HSP90-related gene. Nucleotides shown in lower case facilitate cloning by adding NcoI restriction sites.

The PCR amplified product was treated with NcoI, isolated from a low-melting temperature agarose gel, and cloned into the same restriction enzyme treated binary vector pBI525 (obtained from William Crosby, Plant Biotechnology Institute, Saskatoon, Sask., Canada), resulting in a clone pBI525GLRaV-3hsp90 (Figure 31). A plant expression cassette, the *Eco*RI and *Hind*III fragment of clone pBI525GLRaV-3hsp90, which contains properly engineered CaMV 35S promoters and a Nos 3' untranslated region, was excised and cloned into a similar restriction enzyme digested plant transformation vector, pBin19 (Figure 14) (Clontech Laboratories, Inc.). Two clones, pBin19GLRaV-3hsp90-12-3 and pBin19GLRaV-3hsp90-12-4 that were shown by PCR to contain the proper size of the incomplete HSP90 gene were used to transform the avirulent *A. tumefaciens*, strain LBA4404 via electroporation (Bio-Rad). The potentially transformed *Agrobacterium* was plated on selective media with 75 μg/ml of kanamycin. *Agrobacterium* lines which contain the HSP90 gene sequence were used to transform tobacco (*Nicotiana tobacum* cv.Havana 423) using standard procedures (Horsch et al., Science, 227:1229-1231 (1985)). Kanamycin resistant tobacco plants were analyzed by PCR for the presence of the transgene. Transgenic tobacco plants with the transgene were self pollinated and seed was harvested.

Example 16 - Custom-PCR Engineering of the 43K ORF

The complete sequence of the GLRaV-3 hsp90 gene was reported in Table 4. However, in the present study, using two custom synthesized oligo primers (93-224, tacttatctagaaccATGGAAGCGAGTCGACGACTA (SEQ ID NO:29) and 93-225, tcttgaggatccatggAGAAACATCGTCGCATACTA (SEQ ID NO:30)) and GLRaV-3 dsRNA as template, the incomplete HSP90 related gene sequence was amplified by RT-PCR which added an NcoI restriction enzyme recognition sequence (CCATGG) around the potential translation initiation codon (ATG) and another NcoI site, 29 nt downstream from the translation termination codon (TAA) (Table 14). The PCR amplified fragment was digested

with NcoI, and cloned into the same restriction enzyme treated plant expression vector, pBI525. Under ampicillin selective conditions, hundreds of antibiotic resistant transformants of *E. coli* strain DH5a were generated. Clones derived from five colonies were selected for further analysis. Restriction enzyme mapping (NcoI or BamHI and EcoRV) showed that three out of five clones contained the proper size of the incomplete GLRaV-3 HSP90 sequence. Among them, two clones were engineered in the correct 5'-3' orientation with respect to the CaMV-AMV gene regulatory elements in the plant expression vector, pBI525. A graphical structure in the region of the plant expression cassette of clone pBI525GLRaV-3hsp90-12 is presented in Figure 14.

The GLRaV-3 HSP90 expression cassette was removed from clone pBI525GLRaV-3hsp90-12 by a complete digestion with HindIII and EcoRI and cloned into the similar restriction enzyme treated plant transformation vector pBin19. A clone designated as pBin19GLRaV-3hsp90-12 was then obtained (Figure 14) and was subsequently mobilized into the avirulent *Agrobacterium* strain LBA4404 using a standard electroporation protocol (Bio-Rad). Potentially transformed Agrobacterium cells were then plated on a selective medium (75 μg/ml kanamycin), and antibiotic resistant colonies were analyzed further by PCR with specific synthesized primers (93-224 and 93-225) to see whether or not the incomplete HSP90 gene was still present. After analysis, clone LBA4404/pBin19GLRaV-3hsp90-12 was selected and used to transform tobacco tissues.

Example 17 - Transformation and Characterization of Transgenic Plants

The genetically engineered *A. tumefaciens* strain, LBA4404/pBin19GLRaV-3hsp90-12, was co-cultivated with tobacco leaf discs as described (Horsch (1985)), Potentially transformed tobacco tissues were selected on MS regeneration medium (Murashige et al., Physiologia Plantarum, 15:473-497 (1962)) containing 300 μg/ml of kanamycin. Numerous shoots developed from kanamycin resistant calli in about 6 weeks.

Rooted tobacco plants were obtained following growth of developed shoots on a rooting medium (MS without hormones) containing 300 μg/ml of kanamycin. Eighteen independent, regenerated kanamycin resistant plants were transplanted in a greenhouse and tested for the presence of the HSP90-related gene by PCR. Fourteen out of eighteen selected kanamycin resistant putative transgenic lines were shown to contain a PCR product with the expected size of 1.2 kb.

The genetically engineered *Agrobacterium tumefaciens* strain LBA4404/pBin19GLRaV-3hsp90-12 was also used to transfrom the grapevine rootstock C. 3309 (*Vitis riparia x Vitis rupestris*). Embryogenic calli of C. 3309 were obtained by culturing anthers on MSE medium (Murashige and Skoog salts plus 0.2% sucrose, 1.1 mg/L 2,-4-D, and 0.2 mg/L BA. The medium was adjust to pH 6.5 and 0.8% Noble agar was added. After autoclaving 100 ml M-0654, 100 ml M-0529 and 1 ml vitamin M-3900 were added to the medium. Ater 60 days primary calli were indcued and transferred to hormone-free HMG medium (1/2 Murashige salts with 10 g/L sucrose, 4.6 g/L glycerol and 0.8% Noble agar) for embryogenesis. Calli with globular or heart-shaped embryos were immersed for 15 min. in *A. tumefaciens* LBA4404/pBin19GLRaV-3hsp90-12 suspended in MS liquid medium. The embryos were blotted on filter paper to remove excess liquid and transferred to HMG medium with acetosyringone (100 μM) and kept for 48 hrs. in the dark at 28°C. The calli were then washed 2-3 times in MS liquid medium plus cefotaxime (300 μg/ml) and carbenicillin (200 μg/ml) and transferred to HMG medium with the same antibiotics for 1-2 weeks. Subsequently, the embryogenic calli were transferred to HMG medium containing 20 or 20 μg/ml kanmamycin and 300 μg/ml cefotaxime plus 200 μg/ml carbenicillin to select for transgenic embryos. After being on selective medium for 3-4 months, growing embryos were transferred to HMG, MGC (full-strength MS salts amended with 20 g/L sucrose, 4.6 g/L glycerol, 1 g/L casein hydrolysate and 0.8% Noble agar), or MSE medium with kanamycin. After 4 months germinated embryos were transferred to baby food jars

containing rooting medium (Woody plant medium described by Lloyd and McCown (1981) "Commercially feasible micropropagation of mountain laurel, *Kalmia latifola*, by use of shoot tip culture," *Proc. Intl. Plant Prop. Soc.*30:421-427, supplemented with 0.1 mg/L BA, 3 g/L activated charcoal and 1.5% sucrose. The pH was adjusted to 5.8 and Noble agar was added to 0.7%) . Plantlets with roots were transplatned to pots with artificial soil mix and grown in greenhouses. 88 grapevine plants have cultivated, and several have been shown to contain the 43 kDa protein gene (by PCR).

Using the methods described above, engineering of the incomplete HSP90 gene of GLRaV-3 into plant expression and transformation vectors has been effected. The targeted gene sequence was shown to be integrated into the plant genome by PCR analysis of the putative transgenic tobacco plants. The engineered *A. tumefaciens* strain LBA4404/pBin19GLRaV-3hsp90-12 has been used to transform grapes and tobacco. Furthermore, this plant transformation vector can serve as a model for construction of other GLRaV-3 vectors, such as coat protein, RdRp, and HSP70, for which nucleotide sequences are disclosed herein.

Since the first demonstration of transgenic tobacco plants expressing the coat protein gene of TMV resulted in resistance against TMV infection (Powell-Abel et al., Science, 232:738-743 (1986), which is hereby incorporated by reference), the phenomenon of the coat protein-mediated protection has been observed for over 20 viruses in at least 10 different taxonomic groups in a wide variety of dicotyledonous plant species (Beachy et al., Annu. Rev. Phytopathol., 28:451-74 (1990); Wilson, Proc. Natl. Acad. Sci., USA, 90:3134-3141 (1993)). If gene silencing (or co-suppression) (Finnegan et al., Bio/Technology, 12:883-888 (1994); Flavell, Proc. Natl. Acad. Sci. USA, 91:3490-3496 (1994)) is one of the resistance mechanisms (Lindbo et al., The Plant Cell, 5:1749-1759 (1993); Pang et al., Bio/Technology, 11:819-824 (1993); Smith et al., The Plant Cell, 6:1441-1453 (1994)), then one would expect to generate

transgenic plants expressing any part of a viral genome sequence to protect plants from that virus infection. Thus, in the present study, transgenic plants expressing the 43K ORF (or the incomplete hsp90 gene) are protected from GLRaV-3 infection.

Since tobacco (*Nicotiana tobaccum* cv. Havana 423) is not the host of GLRaV-3, direct evaluation of virus resistance was not possible in tobacco. However, after mechanical inoculation of *N. benthamiana* with grapevine leafroll infected tissue, Boscia (1995) have recovered a long closterovirus from *N. benthamiana* which is probably GLRaV-2. Thus, it is believed that other types of grapevine leafroll associated closteroviruses can also be mechanically transmitted to *N. benthamiana*. With transfer of an engineered 43K ORF from GLRaV-3 to *N. benthamiana* and to the grapevine rootstock Couderc 3309, it is possible to evaluate the resistance of those plants against GLRaV-2 infection.

Example 18 - Coat Protein-mediated Protection and Other Forms of Pathogen-derived Resistance

The successful engineering technique used in the above work can be utilized to engineer other gene sequences of GLRaV-3 which have since been identified. Among these, the coat protein gene of GLRaV-3 is the primary candidate since coat protein-mediated protection (Beachy (1990); Hull et al., Crit. Rev. Plant Sci., 11:17-33 (1992); Wilson (1993)) has been the most successful example in the application of the concept of pathogen-derived resistance (Sanford et al., J. Theor. Biol., 113:395-405 (1985)). Construction of plant expression vector (pEPT8/cpGLRaV-3) and *Agrobacterium* binary vector (pGA482pEPT8/cpGLRaV-3) was done following a strategy similar to the above. The GLRaV-3 coat protein gene was PCR amplified with primers (KSL95-5, actatttctagaaccATGGCATTTGAACTGAAATT (SEQ ID NO:31), and KSL95-6, ttctgaggatccatggTATAAGCTCCCATGAATTAT (SEQ ID NO:32)) and cloned into pEPT8 after NcoI treatment. The expression cassette from pEPT8/cpGLRaV-3 (including double CaMV 35S

enhancers, 35S promotor, alfalfa mosaic virus leader sequence, GLRaV-3 coat protein gene, and 35S terminator) was digested with HindIII and cloned into pGA482G (Figure 15). The resulting *Agrobacterium* binary vector (pGA482GpEPT8/cpGLRaV-3) was mobilized into *A. tumefaciens* strain C58Z707 and used for transformation of grapevines.

Other gene sequences (e.g., ORF 1b, the RNA dependent RNA polymerase) can also be used, as replicase-mediated protection has been effectively used to protect plants from virus infection (Carr et al., Seminars in Virology, 4:339-347 (1993); Golemboski et al., Proc. Natl. Acad. Sci. USA, 87:6311-6315 (1990)). The HSP70 homologue can also be used to generate transgenic plants that are resistant to all types of grapevine leafroll associated closteroviruses because significant sequence similarity is observed over HSP70 conserved domains. Moreover, the phenomenon of RNA-mediated protection has also been observed (de Haan et al., Bio/Technology, 10:1133-1137 (1992); Farinelli et al., Mol. Plant Microbe Interact., 6:284-292 (1993); Kawchuk et al., Mol. Plant Microbe Interact, 4:247-253 (1991); Lindbo et al., Virology, 189:725-733 (1992); Lindbo et al., Mol. Plant Microbe Interact, 5:144-153 (1992); Lindbo et al., Seminars in Virology, 4:369-379 (1993); Pang (1993); Van Der Wilk et al., Plant Mol. Biol., 17:431-440 (1991)). Thus, untranslatable transcript versions of the above mentioned GLRaV-3 genes also produce leafroll resistant transgenic plants.

Another form of pathogen-derived resistance effective in the control of plant viral disease is the use of antisense RNA. Transgenic tobacco plants expressing the antisense sequence of the coat protein gene of cucumber mosaic virus (CMV) showed a delay in symptom expression by CMV infection (Cuozzo et al., Bio/Technology, 6:549-554 (1988)). Transgenic plants expressing either potato virus X (PVX) coat protein or its antisense transcript were protected from infection by PVX. However, plants expressing antisense RNA were protected only at low inoculum concentration. The extent of this protection mediated by antisense transcript is usually lower than

transgenic plants expressing the coat protein (Hemenway et al., EMBO J., 7:1273-1280 (1988)). This type of resistance has also been observed in bean yellow mosaic virus (Hammond et al., Phytopathology, 81:1174 (1991), tobacco etch virus (Lindbo et al., Virology, 189:725-733 (1992)) potato, virus Y (Farinelli (1993)), and zucchini yellow mosaic virus (Fang et al., Mol. Plant Microbe Interact., 6:358-367 (1993)). However, high level of resistance mediated by antisense sequence was observed to be similar to potato plants (Russet Burbank) expressing potato leafroll virus coat protein (Kawchuk (1991)). Besides using antisense transcript of the virus coat protein gene, other virus genome sequences have also been demonstrated to be effective. These included the 51-nucleotide sequences near the 5' end of TMV RNA (Nelson et al., Gene (Abst), 127:227-232 (1993)) and noncoding region of turnip yellow mosaic virus genome (Zaccomer et al., Gene, 87-94 (1993)).

GLRaV-3 has been shown to be transmitted by mealybugs and in some cases it has been shown to spread rapidly in vineyards (see hereinabove). This disease will become more of a problem if mealybugs become resistant to insecticides or if insecticide use is restricted. Thus, the development of leafroll resistant grapevines is environmentally sound and good for the economics of grape growing.

Although grapevine is a natural host of *Agrobacterium* (*A. vitis* is the causal agent of the grapevine crown gall disease), transformation of grapevine has proven difficult (Baribault et al., J. Exp. Bot., 41:1045-1049 (1990); Baribault et al., Plant Cell Reports, 8:137-140 (1989); Colby et al., J. Am. Soc. Hort. Sci., 116:356-361 (1991); Guellec et al., Plant Cell Tissue Organ Cult., 20:211-216 (1990); Hebert et al., Plant Cell Reports, 12:585-589 (1993); Le Gall et al., Plant Science, 102:161-170 (1994); Martinelli et al., Theor. Appl. Genetics, 88:621-628 (1994); Mullins et al., Bio/Technology, 8:1041-1045 (1990)). Recently, an efficient regeneration system using proliferative somatic embryogenesis and subsequent plant development has been developed from

zygotic embryos of stenospermic seedless grapes (Mozsar, J. et al., Vitis, 33:245-246 (1994); Emerschad (1995)). Using this regeneration system, Scorza et al., Plant Cell Reports, 14:589-592 (1995) succeeded in obtaining transgenic grapevines through zygotic-derived somatic embryos after particle-wounding/*A. tumefaciens* treatment. Using a Biolistic device, tiny embryos were shot with gold particles (1.0 μm in diameter). The wounded embryos were then co-cultivated with *A. tumefaciens* containing engineered plasmids carrying the selection marker of kanamycin resistance and β-glucuronidase (GUS) genes. Selection of transgenic grapevines was carried out with 20 μg/ml kanamycin in the initial stage and then 40 μg/ml for later proliferation. Small rooted seedlings were obtained from embryogenic culture within 5 months of bombardment/*A. tumefaciens* (Scorza (1995)). Transgenic grapevines were analyzed by PCR and Southern hybridization, and shown to carry the transgenes. The above-mentioned grapevine transformation approach has been carried out in the current investigation to generate transgenic grapevines expressing GLRaV-3 genes. Evaluation of any potential leafroll resistance on transgenic grapevines is carried out using insect vectors or by grafting.

Example 19 - Production of Antibodies Recognizing GLRaV3

E. coli harboring the clone pCP10-1, which contains the major portion of the coat protein gene of GLRaV3 (Figure 4), was used to express the coat protein and the β-galactosidase fusion protein. About 500 ml of LB medium containing 50 μg/ml of ampicillin was inoculated with a single colony and incubated with rigorous shaking for overnight until log-phase growth. Expression of the fusion protein was induced by the addition of 1 mM IPTG. Bacteria were harvested by centrifugation at 5,000 rpm for 10 min. The bacterial envelope was broken by sonication. After low speed centrifugation to remove cell debris, the fusion protein was precipitated by the addition of saturated ammonium sulfate, then resuspended in PBS buffer and electrophoresed in a SDS-

polyacrylamide gel (SDS-PAGE). The fusion protein band was excised after soaking the SDS-PAGE gel in 0.25M KCl to locate the protein band. The protein was eluted with buffer (0.05M Tris-HCl, pH 7.9, 0.1% SDS, 0.1 mM EDT and 0.15 M NaCl) and precipitated by the addition of trichloroacetic acid to a final concentration of 20%.

An antiserum was prepared by immunization of a rabbit with 0.5-1 mg of the purified protein emulsified with Freund's completed adjuvant followed by two more weekly injections of 0.5-1 mg protein emulsified with Freund's incomplete adjuvant. After the last injection, antiserum was prepared from blood collected from the rabbit every week for a period of 4 months.

On Western blot analysis, the antibody gave a specific reaction to the 41K protein in GLRaV3 infected tissue as well as to the fusion protein itself (50K) and generated a pattern similar to the pattern seen in Figure 3. This antibody was also successfully used as a coating antibody and as an antibody-conjugate in an enzyme linked immunosorbent assay (ELISA).

The above method of producing antibody to GLRaV3 can also be applied to other GLRaV-3 gene sequences of the present invention. The method affords a large amount of highly purified protein from *E. coli* from which antibodies can be readily obtained. It is particularly useful in the common case where it is rather difficult to obtain sufficient amount of purified virus from GLRaV3 infected grapevine tissues.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Cornell Research Foundation, Inc.

(ii) TITLE OF INVENTION: Grapevine Leafroll Virus Proteins and Their Uses

(iii) NUMBER OF SEQUENCES: 32

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Greenlee, Winner and Sullivan, P.C.
(B) STREET: 5370 Manhattan Circle, Suite 201
(C) CITY: Boulder
(D) STATE: Colorado
(E) COUNTRY: US
(F) ZIP: 80303

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/009,008
(B) FILING DATE: 20-DEC-1995

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Ferber, Donna M.
(B) REGISTRATION NUMBER: 33,878
(C) REFERENCE/DOCKET NUMBER: 99-96 ZA

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (303) 499-8080
(B) TELEFAX: (303) 499-8089

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4173 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TCCAGGAGGT TACGTTCGAG TCGTTTGCGC GTATTTTCTA GGGCTATTGT GGAGGATACG 240

ATCAAGGTTA TGAAGGGCAT GAAATCAGAG GATGGTAAAC CACTCCCTAT AGCCGAGGAT 300

TCCGTGTACG CGTTCATGAC AGGCAATATG TCAAACGTTC ATTGCACTAG GGCTGGTTTG 360

CTCGGGGGCT CAAAGGCTTG CGCGGCTTCT TTAGCTGTGA AGGGTGCAGC TTCACGCGCT 420

ACTGGAACAA AACTCTTTTC AGGTCTCACA TCCTTTCTTT CCGCCGGTGG TCTGTTTTAC 480

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AATTCACCTG TAGGCCTCTT AGAACCTGGA GCTTCGGTTG CGAAGCGGGT CGTTTCCGGA 600

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AGGGTGCTTA TTGGTGTTTT TACTCTTTCC ATGGCTCTCA CTCCGGTGGT CTGGAAGTAC 720

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GCGTTAACAG TGACTCGAGG AGGGCTATCT TCGGCGGTTG CGGTGACCAG AAATACAGTG 900

GCTAGGCGTC AGGTACCATT GGCGTTGCTT TCGTTTTCCA CGTCTTACGC AGTCAGTGGT 960

TGCACTTTGT TAGGTATTTG GGCTCATGCT CTCCCTAGGC ATTTGATGTT CTTCTTTGGC 1020

CTAGGGACGC TCTTCGGGGT GAGTGCCAGT ACCAATTCTT GGTCGCTTGG GGGCTATACG 1080

AACAGTCTGT TCACCGTACC GGAATTAACT TGGGAAGGGA GGAGTTACAG ATCTTTATTG 1140

CCCCAAGCAG CTTTAGGTAT TTCTCTCGTT GTGCGCGGGT TGTTAAGTGA AACTGTGCCA 1200

CAACTAACGT ACGTACCGCC GATTGAAGGT CGGAATGTTT ATGATCAGGC ACTAAATTTT 1260

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GCTGAAGTTG AAAAGGATCT AATAACACCA CTTGGTACAG CTGTCTTAGA GTCGCCCCCC 1560

GTAGGTCCTG AAGCTGGGAG CGCGCCCAAC GTCGAGGACG GTTGTCCGGA GGTTGAAGCT 1620

GAGAAATGTT CGGAGGTCAT CGTTGACGTT CCTAGTTCAG AACCGCCGGT ACAAGAAGTC 1680

CTTGAATCAA CCAATGGTGT CCAAGCTGCA AGAACTGAAG AGGTTGTGCA GGGCGACACA 1740

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GCACATGAAG CTGGTCTTGA GGCATCTAGT GGCGCGGTCG TGGAGCCATT GCAAGTTTCT 1860

GTGCCAGTAG CCGTAGAGAA AACTGTTTTA TCTGTCGAGA AGGCGCGTGA GCTAAAGGCG 1920

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TTACCACGAG GGGCTCTAAA AATTAGTGAG GATACCGTTC GTAAGGAATT GTGCATGTTT 2040

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TTCTCAAATG CGTTTACCTT TGTCGATAGC TTGAAAGGGA GGAGTGCGGT CTTTTTCTCA 2160

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CAGAAGTACA AGATGGGTGG AGGCGTACCA TTCCACGCTG ATGACGAGGA GTGCTATCCA 2340

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ATTAATTTGA TGGGTGCACT GAAAGTTGCT CGATGTGGTA TGGTGGGTGA ATCATTTAAG 3240

TCGTTCGAAT ACAAATGCTA TAATGCTCCC CCAGGTGGCG GTAAGACGAC GATGCTAGTG 3300

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GCGTCGGAAG GCCTCTTTTT TGGAGACATA AATCAGATAC CATTCATAAA CCGGGAGAAG 3600

GTGTTTAGGA TGGATTGTGC TGTATTTGTT CCAAAGAAGG AAAGCGTTGT ATACACTTCT 3660

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GTCGGCACAT ATATTAGCGA CGCGTCGCCT CAATCAGTAT CCGACGCTTT GCTTCACACG 4140

TTCGCCCCGG CTGGTTGCTT TCGAGGTATA TGA 4173

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1390 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Val Ser Thr Tyr Ala Lys Ser Val Met Asn Asp Asn Phe Asn Ile Leu
1 5 10 15

Glu Thr Leu Val Thr Leu Pro Lys Ser Phe Ile Val Lys Val Pro Gly
20 25 30

Ser Val Leu Val Ser Ile Thr Thr Ser Gly Ile Ser Asp Lys Leu Glu
35 40 45

Leu Arg Gly Ala Phe Asp Val Ser Lys Lys Asn Phe Ser Arg Arg Leu
50 55 60

Arg Ser Ser Arg Leu Arg Val Phe Ser Arg Ala Ile Val Glu Asp Thr
65 70 75 80

Ile Lys Val Met Lys Gly Met Lys Ser Glu Asp Gly Lys Pro Leu Pro
85 90 95

Ile Ala Glu Asp Ser Val Tyr Ala Phe Met Thr Gly Asn Met Ser Asn
100 105 110

Val His Cys Thr Arg Ala Gly Leu Leu Gly Gly Ser Lys Ala Cys Ala
115 120 125

Ala Ser Leu Ala Val Lys Gly Ala Ala Ser Arg Ala Thr Gly Thr Lys
130 135 140

Leu Phe Ser Gly Leu Thr Ser Phe Leu Ser Ala Gly Gly Leu Phe Tyr
145 150 155 160

Asp Glu Gly Leu Thr Pro Gly Glu Arg Leu Asp Ala Leu Thr Arg Arg
165 170 175

Glu His Ala Val Asn Ser Pro Val Gly Leu Leu Glu Pro Gly Ala Ser
180 185 190

Val Ala Lys Arg Val Val Ser Gly Thr Lys Ala Phe Leu Ser Glu Leu
195 200 205

Ser Leu Glu Asp Phe Thr Thr Phe Val Ile Lys Asn Arg Val Leu Ile
210 215 220

Gly Val Phe Thr Leu Ser Met Ala Leu Thr Pro Val Val Trp Lys Tyr
225 230 235 240

Arg Arg Asn Ile Ala Arg Thr Gly Val Asp Val Phe His Arg Ala Arg
245 250 255

Ser Gly Thr Ala Ala Ile Gly Leu Gln Cys Leu Ser Gly Gly Arg Ser
260 265 270

Leu Ala Gly Asp Ala Ala Arg Gly Ala Leu Thr Val Thr Arg Gly Gly
275 280 285

Leu Ser Ser Ala Val Ala Val Thr Arg Asn Thr Val Ala Arg Arg Gln
290 295 300

Val Pro Leu Ala Leu Leu Ser Phe Ser Thr Ser Tyr Ala Val Ser Gly
305 310 315 320

Cys Thr Leu Leu Gly Ile Trp Ala His Ala Leu Pro Arg His Leu Met
325 330 335

Phe Phe Phe Gly Leu Gly Thr Leu Phe Gly Val Ser Ala Ser Thr Asn
340 345 350

Ser Trp Ser Leu Gly Gly Tyr Thr Asn Ser Leu Phe Thr Val Pro Glu
355 360 365

Leu Thr Trp Glu Gly Arg Ser Tyr Arg Ser Leu Leu Pro Gln Ala Ala
370 375 380

Leu Gly Ile Ser Leu Val Val Arg Gly Leu Leu Ser Glu Thr Val Pro
385 390 395 400

Gln Leu Thr Tyr Val Pro Pro Ile Glu Gly Arg Asn Val Tyr Asp Gln
405 410 415

Ala Leu Asn Phe Tyr Arg Asp Phe Asp Tyr Asp Asp Gly Ala Gly Pro
420 425 430

Ser Gly Thr Ala Gly Gln Ser Asp Pro Gly Thr Asn Thr Ser Asp Thr
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Ser Ser Val Phe Ser Asp Asp Gly Leu Pro Ala Ser Gly Gly Gly Phe
450 455 460

Asp Ala Arg Val Glu Ala Gly Pro Ser His Ala Val Asp Glu Ser Pro
465 470 475 480

Arg Gly Ser Val Glu Phe Val Tyr Arg Glu Arg Val Asp Glu His Pro
485 490 495

Ala Cys Gly Glu Ala Glu Val Glu Lys Asp Leu Ile Thr Pro Leu Gly
500 505 510

Thr Ala Val Leu Glu Ser Pro Pro Val Gly Pro Glu Ala Gly Ser Ala
515 520 525

Pro Asn Val Glu Asp Gly Cys Pro Glu Val Glu Ala Glu Lys Cys Ser
530 535 540

Glu Val Ile Val Asp Val Pro Ser Ser Glu Pro Pro Val Gln Glu Val
545 550 555 560

Leu Glu Ser Thr Asn Gly Val Gln Ala Ala Arg Thr Glu Glu Val Val
565 570 575

Gln Gly Asp Thr Cys Gly Ala Gly Val Ala Lys Ser Glu Val Ser Gln
580 585 590

Arg Val Phe Pro Ala Gln Val Pro Ala His Glu Ala Gly Leu Glu Ala
595 600 605

Ser Ser Gly Ala Val Val Glu Pro Leu Gln Val Ser Val Pro Val Ala
610 615 620

Val Glu Lys Thr Val Leu Ser Val Glu Lys Ala Arg Glu Leu Lys Ala
625 630 635 640

Val Asp Lys Gly Lys Ala Val Val His Ala Lys Glu Val Lys Asn Val
645 650 655

Pro Val Lys Thr Leu Pro Arg Gly Ala Leu Lys Ile Ser Glu Asp Thr
660 665 670

Val Arg Lys Glu Leu Cys Met Phe Arg Thr Cys Ser Cys Gly Val Gln
675 680 685

Leu Asp Val Tyr Asn Glu Ala Thr Ile Ala Thr Arg Phe Ser Asn Ala
690 695 700

Phe Thr Phe Val Asp Ser Leu Lys Gly Arg Ser Ala Val Phe Phe Ser
705 710 715 720

Lys Leu Gly Glu Gly Tyr Thr Tyr Asn Gly Gly Ser His Val Ser Ser
725 730 735

Gly Trp Pro Arg Ala Leu Glu Asp Ile Leu Thr Ala Ile Lys Tyr Pro
740 745 750

Ser Val Phe Asp His Cys Leu Val Gln Lys Tyr Lys Met Gly Gly Gly
755 760 765

Val Pro Phe His Ala Asp Asp Glu Glu Cys Tyr Pro Ser Asp Asn Pro
770 775 780

Ile Leu Thr Val Asn Leu Val Gly Lys Ala Asn Phe Ser Thr Lys Cys
785 790 795 800

Arg Lys Gly Gly Lys Val Met Val Ile Asn Val Ala Ser Gly Asp Tyr
805 810 815

Phe Leu Met Pro Cys Gly Phe Gln Arg Thr His Leu His Ser Val Asn
820 825 830

Ser Ile Asp Glu Gly Arg Ile Ser Leu Thr Phe Arg Ala Thr Arg Arg
835 840 845

Val Phe Gly Val Gly Arg Met Leu Gln Leu Ala Gly Gly Val Ser Asp
850 855 860

Glu Lys Ser Pro Gly Val Pro Asn Gln Gln Pro Gln Ser Gln Gly Ala
865 870 875 880

Thr Arg Thr Ile Thr Pro Lys Ser Gly Gly Lys Ala Leu Ser Glu Gly
885 890 895

Ser Gly Arg Glu Val Lys Gly Arg Ser Thr Tyr Ser Ile Trp Cys Glu
900 905 910

Gln Asp Tyr Val Arg Lys Cys Glu Trp Leu Arg Ala Asp Asn Pro Val
915 920 925

Met Ala Leu Lys Pro Gly Tyr Thr Pro Met Thr Phe Glu Val Val Lys
930 935 940

Ala Gly Thr Ser Glu Asp Ala Val Val Glu Tyr Leu Lys Tyr Leu Ala
945 950 955 960

Ile Gly Ile Gly Arg Thr Tyr Arg Ala Leu Leu Met Ala Arg Asn Ile
965 970 975

Ala Val Thr Thr Ala Glu Gly Val Leu Lys Val Pro Asn Gln Val Tyr
980 985 990

Glu Ser Leu Pro Gly Phe His Val Tyr Lys Ser Gly Thr Asp Leu Ile
995 1000 1005

Phe His Ser Thr Gln Asp Gly Leu Arg Val Arg Asp Leu Pro Tyr Val
1010 1015 1020

Phe Ile Ala Glu Lys Gly Ile Phe Ile Lys Gly Lys Asp Val Asp Ala
1025 1030 1035 1040

Val Val Ala Leu Gly Asp Asn Leu Ser Val Cys Asp Asp Ile Leu Val
1045 1050 1055

Phe His Asp Ala Ile Asn Leu Met Gly Ala Leu Lys Val Ala Arg Cys
1060 1065 1070

Gly Met Val Gly Glu Ser Phe Lys Ser Phe Glu Tyr Lys Cys Tyr Asn
1075 1080 1085

Ala Pro Pro Gly Gly Gly Lys Thr Thr Met Leu Val Asp Glu Phe Val
1090 1095 1100

Lys Ser Pro Asn Ser Thr Ala Thr Ile Thr Ala Asn Val Gly Ser Ser
1105 1110 1115 1120

Glu Asp Ile Asn Met Ala Val Lys Lys Arg Asp Pro Asn Leu Glu Gly
1125 1130 1135

Leu Asn Ser Ala Thr Thr Val Asn Ser Arg Val Val Asn Phe Ile Val
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Arg Gly Met Tyr Lys Arg Val Leu Val Asp Glu Val Tyr Met Met His
1155 1160 1165

Gln Gly Leu Leu Gln Leu Gly Val Phe Ala Thr Gly Ala Ser Glu Gly
1170 1175 1180

Leu Phe Phe Gly Asp Ile Asn Gln Ile Pro Phe Ile Asn Arg Glu Lys
1185 1190 1195 1200

Val Phe Arg Met Asp Cys Ala Val Phe Val Pro Lys Lys Glu Ser Val
1205 1210 1215

Val Tyr Thr Ser Lys Ser Tyr Arg Cys Pro Leu Asp Val Cys Tyr Leu
1220 1225 1230

Leu Ser Ser Met Thr Val Arg Gly Thr Glu Lys Cys Tyr Pro Glu Lys
1235 1240 1245

Val Val Ser Gly Lys Asp Lys Pro Val Val Arg Ser Leu Ser Lys Arg
1250 1255 1260

Pro Ile Gly Thr Thr Asp Asp Val Ala Glu Ile Asn Ala Asp Val Tyr
1265 1270 1275 1280

Leu Cys Met Thr Gln Leu Glu Lys Ser Asp Met Lys Arg Ser Leu Lys
1285 1290 1295

Gly Lys Gly Lys Glu Thr Pro Val Met Thr Val His Glu Ala Gln Gly
1300 1305 1310

Lys Thr Phe Ser Asp Val Val Leu Phe Arg Thr Lys Lys Ala Asp Asp
1315 1320 1325

Ser Leu Phe Thr Lys Gln Pro His Ile Leu Val Gly Leu Ser Arg His
1330 1335 1340

Thr Arg Ser Leu Val Tyr Ala Ala Leu Ser Ser Glu Leu Asp Asp Lys
1345 1350 1355 1360

Val Gly Thr Tyr Ile Ser Asp Ala Ser Pro Gln Ser Val Ser Asp Ala
1365 1370 1375

Leu Leu His Thr Phe Ala Pro Ala Gly Cys Phe Arg Gly Ile
1380 1385 1390

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1602 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGAATTTTG GACCGACCTT CGAAGGGGAG TTGGTACGGA AGATACCAAC AAGTCATTTT 60

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TTCTTTGAGG ATGATTTCGA AACTTCAGAT CAGTCTTTCC TCATAGAAGA TGTGCGCATT 180

TCTGAATCTT TTTCTCATTT TGCGTCGAAA ATAGAGGATA GGTTTTACAG TTTTATTAGG 240

TCTAGCGTAG GTTTACCAAA GCGCAACACC TTGAAGTGTA ACCTCGTCAC GTTTGAAAAT 300

AGGAATTCCA ACGCCGATCG CGGTTGTAAC GTGGGTTGTG ACGACTCTGT GGCGCATGAA 360

CTGAAGGAGA TTTTCTTCGA GGAGGTCGTT AACAAAGCTC GTTTAGCAGA GGTGACGGAA 420

AGCCATTTGT CCAGCAACAC GATGTTGTTA TCAGATTGGT TGGACAAAAG GGCACCTAAC 480

GCTTACAAGT CTCTCAAGCG GGCTTTAGGT TCGGTTGTCT TTCATCCGTC TATGTTGACG 540

TCTTATACGC TCATGGTGAA AGCAGACGTA AAACCCAAGT TGGACAATAC GCCATTGTCG 600

AAGTACGTAA CGGGGCAGAA TATAGTCTAC CACGATAGGT GCGTAACTGC GCTTTTTTCT 660

TGCATTTTTA CTGCGTGCGT AGAGCGCTTA AAATACGTAG TGGACGAAAG GTGGCTCTTC 720

TACCACGGGA TGGACACTGC GGAGTTGGCG GCTGCATTGA GGAACAATTT GGGGGACATC 780

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TTGTGCACCT TGTTGTCCGT AGTACTTAGG GGATTAGATT ATAGTTATAT TGTAGTTAGC 1080

GGTGATGATA GCCTTATATT TAGTCGGCAG CCGTTGGATA TTGATACGTC GGTTCTGAGC 1140

GATAATTTTG GTTTTGACGT AAAGATTTTT AACCAAGCTG CTCCATATTT TTGTTCTAAG 1200

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GTCGATCTTT CGAAGGGTTT CAATAGAGAG GACGTCATCC AGGAATTAGC TAAGCTGGTG 1380

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CGCCCTATTC AGAGGACCGA GTCGCTTTCC TTGCTGGCCT TGAAGGCAAG AATTTTAAGG 1560

TGGAAAGCTT CTCGTTTTGC CTTTTCGATA AAGAGGGGTT AA 1602

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 533 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Phe Gly Pro Thr Phe Glu Gly Glu Leu Val Arg Lys Ile Pro
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Thr Ser His Phe Val Ala Val Asn Gly Phe Leu Glu Asp Leu Leu Asp
20 25 30

Gly Cys Pro Ala Phe Asp Tyr Asp Phe Phe Glu Asp Asp Phe Glu Thr
35 40 45

Ser Asp Gln Ser Phe Leu Ile Glu Asp Val Arg Ile Ser Glu Ser Phe
50 55 60

Ser His Phe Ala Ser Lys Ile Glu Asp Arg Phe Tyr Ser Phe Ile Arg
65 70 75 80

Ser Ser Val Gly Leu Pro Lys Arg Asn Thr Leu Lys Cys Asn Leu Val
85 90 95

Thr Phe Glu Asn Arg Asn Ser Asn Ala Asp Arg Gly Cys Asn Val Gly
100 105 110

Cys Asp Asp Ser Val Ala His Glu Leu Lys Glu Ile Phe Phe Glu Glu
115 120 125

Val Val Asn Lys Ala Arg Leu Ala Glu Val Thr Glu Ser His Leu Ser
130 135 140

Ser Asn Thr Met Leu Leu Ser Asp Trp Leu Asp Lys Arg Ala Pro Asn
145 150 155 160

Ala Tyr Lys Ser Leu Lys Arg Ala Leu Gly Ser Val Val Phe His Pro
165 170 175

Ser Met Leu Thr Ser Tyr Thr Leu Met Val Lys Ala Asp Val Lys Pro
180 185 190

Lys Leu Asp Asn Thr Pro Leu Ser Lys Tyr Val Thr Gly Gln Asn Ile
195 200 205

Val Tyr His Asp Arg Cys Val Thr Ala Leu Phe Ser Cys Ile Phe Thr
210 215 220

Ala Cys Val Glu Arg Leu Lys Tyr Val Val Asp Glu Arg Trp Leu Phe
225 230 235 240

Tyr His Gly Met Asp Thr Ala Glu Leu Ala Ala Ala Leu Arg Asn Asn
245 250 255

Leu Gly Asp Ile Arg Gln Tyr Tyr Thr Tyr Glu Leu Asp Ile Ser Lys
260 265 270

Tyr Asp Lys Ser Gln Ser Ala Leu Met Lys Gln Val Glu Glu Leu Ile
275 280 285

Leu Leu Thr Leu Gly Val Asp Arg Glu Val Leu Ser Thr Phe Phe Cys
290 295 300

Gly Glu Tyr Asp Ser Val Val Arg Thr Met Thr Lys Glu Leu Val Leu
305 310 315 320

Ser Val Gly Ser Gln Arg Arg Ser Gly Gly Ala Asn Thr Trp Leu Gly
325 330 335

Asn Ser Leu Val Leu Cys Thr Leu Leu Ser Val Val Leu Arg Gly Leu
340 345 350

Asp Tyr Ser Tyr Ile Val Val Ser Gly Asp Asp Ser Leu Ile Phe Ser
355 360 365

Arg Gln Pro Leu Asp Ile Asp Thr Ser Val Leu Ser Asp Asn Phe Gly
370 375 380

Phe Asp Val Lys Ile Phe Asn Gln Ala Ala Pro Tyr Phe Cys Ser Lys
385 390 395 400

Phe Leu Val Gln Val Glu Asp Ser Leu Phe Phe Val Pro Asp Pro Leu
405 410 415

Lys Leu Phe Val Lys Phe Gly Ala Ser Lys Thr Ser Asp Ile Asp Leu
420 425 430

Leu His Glu Ile Phe Gln Ser Phe Val Asp Leu Ser Lys Gly Phe Asn
435 440 445

Arg Glu Asp Val Ile Gln Glu Leu Ala Lys Leu Val Thr Arg Lys Tyr
450 455 460

Lys His Ser Gly Trp Thr Tyr Ser Ala Leu Cys Val Leu His Val Leu
465 470 475 480

Ser Ala Asn Phe Ser Gln Phe Cys Arg Leu Tyr Tyr His Asn Ser Val
485 490 495

Asn Leu Asp Val Arg Pro Ile Gln Arg Thr Glu Ser Leu Ser Leu Leu
500 505 510

Ala Leu Lys Ala Arg Ile Leu Arg Trp Lys Ala Ser Arg Phe Ala Phe
515 520 525

Ser Ile Lys Arg Gly
530

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1650 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGGAAGTAG GTATAGATTT TGGAACCACT TTCAGCACAA TCTGCTTTTC CCCATCTGGG 60

GTCAGCGGTT GTACTCCTGT GGCCGGTAGT GTTTACGTTG AAACCCAAAT TTTTATACCT 120

GAAGGTAGCA GTACTTACTT AATTGGTAAA GCTGCGGGGA AAGCTTATCG TGACGGTGTA 180

GAGGGAAGGT TGTATGTTAA CCCGAAAAGG TGGGCAGGTG TGACGAGGGA TAACGTCGAA 240

CGCTACGTCG AGAAATTAAA ACCTACATAC ACCGTGAAGA TAGACAGCGG AGGCGCCTTA 300

TTAATTGGAG GTTTAGGTTC CGGACCAGAC ACCTTATTGA GGGTCGTTGA CGTAATATGT 360

TTATTCTTGA GAGCCTTGAT ACTGGAGTGC GAAAGGTATA CGTCTACGAC GGTTACAGCA 420

GCTGTTGTAA CGGTACCGGC TGACTATAAC TCCTTTAAAC GAAGCTTCGT TGTTGAGGCG 480

CTAAAAGGTC TTGGTATACC GGTTAGAGGT GTTGTTAACG AACCGACGGC CGCAGCCCTC 540

TATTCCTTAG CTAAGTCGCG AGTAGAAGAC CTATTATTAG CGGTTTTTGA TTTTGGGGGA 600

GGGACTTTCG ACGTCTCATT CGTTAAGAAG AAGGGAAATA TACTATGCGT CATCTTTTCA 660

GTGGGTGATA ATTTCTTGGG TGGTAGAGAT ATTGATAGAG CTATCGTGGA AGTTATCAAA 720

CAAAAGATCA AAGGAAAGGC GTCTGATGCC AAGTTAGGGA TATTCGTATC CTCGATGAAG 780

GAAGACTTGT CTAACAATAA CGCTATAACG CAACACCTTA TCCCCGTAGA AGGGGGTGTG 840

GAGGTTGTGG ATTTGACTAG CGACGAACTG GACGCAATCG TTGCACCATT CAGCGCTAGG 900

GCTGTGGAAG TATTCAAAAC TGGTCTTGAC AACTTTTACC CAGACCCGGT TATTGCCGTT 960

ATGACTGGGG GGTCAAGTGC TCTAGTTAAG GTCAGGAGTG ATGTGGCTAA TTTGCCGCAG 1020

ATATCTAAAG TCGTGTTCGA CAGTACCGAT TTTAGATGTT CGGTGGCTTG TGGGGCTAAG 1080

GTTTACTGCG ATACTTTGGC AGGTAATAGC GGACTGAGAC TGGTGGACAC TTTAACGAAT 1140

ACGCTAACGG ACGAGGTAGT GGGTCTTCAG CCGGTGGTAA TTTTCCCGAA AGGTAGTCCA 1200

ATACCCTGTT CATATACTCA TAGATACACA GTGGGTGGTG GAGATGTGGT ATACGGTATA 1260

TTTGAAGGGG AGAATAACAG AGCTTTTCTA AATGAGCCGA CGTTCCGGGG CGTATCGAAA 1320

CGTAGGGGAG ACCCAGTAGA GACCGACGTG GCGCAGTTTA ATCTCTCCAC GGACGGAACG 1380

GTGTCTGTTA TCGTTAATGG TGAGGAAGTA AAGAATGAAT ATCTGGTACC CGGGACAACA 1440

AACGTACTGG ATTCATTGGT CTATAAATCT GGGAGAGAAG ATTTAGAGGC TAAGGCAATA 1500

CCAGAGTACT TGACCACACT GAATATTTTG CACGATAAGG CTTTCACGAG GAGAAACCTG 1560

GGTAACAAAG ATAAGGGGTT CTCGGATTTA AGGATAGAAG AAAATTTTTT AAAATCCGCC 1620

GTAGATACAG ACACGATTTT GAATGGATAA 1650

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 549 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Val Gly Ile Asp Phe Gly Thr Thr Phe Ser Thr Ile Cys Phe
1 5 10 15

Ser Pro Ser Gly Val Ser Gly Cys Thr Pro Val Ala Gly Ser Val Tyr
20 25 30

Val Glu Thr Gln Ile Phe Ile Pro Glu Gly Ser Ser Thr Tyr Leu Ile
35 40 45

Gly Lys Ala Ala Gly Lys Ala Tyr Arg Asp Gly Val Glu Gly Arg Leu
50 55 60

Tyr Val Asn Pro Lys Arg Trp Ala Gly Val Thr Arg Asp Asn Val Glu
65 70 75 80

Arg Tyr Val Glu Lys Leu Lys Pro Thr Tyr Thr Val Lys Ile Asp Ser
85 90 95

Gly Gly Ala Leu Leu Ile Gly Gly Leu Gly Ser Gly Pro Asp Thr Leu
100 105 110

Leu Arg Val Val Asp Val Ile Cys Leu Phe Leu Arg Ala Leu Ile Leu
115 120 125

Glu Cys Glu Arg Tyr Thr Ser Thr Thr Val Thr Ala Ala Val Val Thr
130 135 140

Val Pro Ala Asp Tyr Asn Ser Phe Lys Arg Ser Phe Val Val Glu Ala
145 150 155 160

Leu Lys Gly Leu Gly Ile Pro Val Arg Gly Val Val Asn Glu Pro Thr
165 170 175

Ala Ala Ala Leu Tyr Ser Leu Ala Lys Ser Arg Val Glu Asp Leu Leu
180 185 190

Leu Ala Val Phe Asp Phe Gly Gly Gly Thr Phe Asp Val Ser Phe Val
195 200 205

Lys Lys Lys Gly Asn Ile Leu Cys Val Ile Phe Ser Val Gly Asp Asn
210 215 220

Phe Leu Gly Gly Arg Asp Ile Asp Arg Ala Ile Val Glu Val Ile Lys
225 230 235 240

Gln Lys Ile Lys Gly Lys Ala Ser Asp Ala Lys Leu Gly Ile Phe Val
245 250 255

Ser Ser Met Lys Glu Asp Leu Ser Asn Asn Asn Ala Ile Thr Gln His
260 265 270

Leu Ile Pro Val Glu Gly Gly Val Glu Val Val Asp Leu Thr Ser Asp
275 280 285

Glu Leu Asp Ala Ile Val Ala Pro Phe Ser Ala Arg Ala Val Glu Val
290 295 300

Phe Lys Thr Gly Leu Asp Asn Phe Tyr Pro Asp Pro Val Ile Ala Val
305 310 315 320

Met Thr Gly Gly Ser Ser Ala Leu Val Lys Val Arg Ser Asp Val Ala
325 330 335

Asn Leu Pro Gln Ile Ser Lys Val Val Phe Asp Ser Thr Asp Phe Arg
340 345 350

Cys Ser Val Ala Cys Gly Ala Lys Val Tyr Cys Asp Thr Leu Ala Gly
355 360 365

Asn Ser Gly Leu Arg Leu Val Asp Thr Leu Thr Asn Thr Leu Thr Asp
370 375 380

Glu Val Val Gly Leu Gln Pro Val Val Ile Phe Pro Lys Gly Ser Pro
385 390 395 400

Ile Pro Cys Ser Tyr Thr His Arg Tyr Thr Val Gly Gly Gly Asp Val
405 410 415

Val Tyr Gly Ile Phe Glu Gly Glu Asn Asn Arg Ala Phe Leu Asn Glu
420 425 430

Pro Thr Phe Arg Gly Val Ser Lys Arg Arg Gly Asp Pro Val Glu Thr
435 440 445

Asp Val Ala Gln Phe Asn Leu Ser Thr Asp Gly Thr Val Ser Val Ile
450 455 460

Val Asn Gly Glu Glu Val Lys Asn Glu Tyr Leu Val Pro Gly Thr Thr
465 470 475 480

Asn Val Leu Asp Ser Leu Val Tyr Lys Ser Gly Arg Glu Asp Leu Glu
485 490 495

Ala Lys Ala Ile Pro Glu Tyr Leu Thr Thr Leu Asn Ile Leu His Asp
500 505 510

Lys Ala Phe Thr Arg Arg Asn Leu Gly Asn Lys Asp Lys Gly Phe Ser
515 520 525

Asp Leu Arg Ile Glu Glu Asn Phe Leu Lys Ser Ala Val Asp Thr Asp
530 535 540

Thr Ile Leu Asn Gly
545

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1452 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGATAAAT ATATTTATGT AACGGGGATA TTAAACCCTA ACGAGGCTAG AGACGAGGTA 60

TTCTCGGTAG TGAATAAGGG ATATATTGGA CCGGGAGGGC GCTCCTTTTC GAATCGTGGT 120

AGTAAGTACA CCGTCGTCTG GGAAAACTCT GCTGCGAGGA TTAGTGGATT TACGTCGACT 180

TCGCAATCTA CGATAGATGC TTTCGCGTAT TTCTTGTTGA AAGGCGGATT GACTACCACG 240

CTCTCTAACC CAATAAACTG TGAGAATTGG GTCAGGTCAT CTAAGGATTT AAGCGCGTTT 300

TTCAGGACCC TAATTAAAGG TAAGATTTAT GCATCGCGTT CTGTGGACAG CAATCTTCCA 360

AAGAAAGACA GGGATGACAT CATGGAAGCG AGTCGACGAC TATCGCCATC GGACGCCGCC 420

TTTTGCAGAG CAGTGTCGGT TCAGGTAGGG AAGTATGTGG ACGTAACGCA GAATTTAGAA 480

AGTACGATCG TGCCGTTAAG AGTTATGGAA ATAAAGAAAA GACGAGGATC AGCACATGTT 540

AGTTTACCGA AGGTGGTATC CGCTTACGTA GATTTTTATA CGAACTTGCA GGAATTGCTG 600

TCGGATGAAG TAACTAGGGC CAGAACCGAT ACAGTTTCGG CATACGCTAC CGACTCTATG 660

GCTTTCTTAG TTAAGATGTT ACCCCTGACT GCTCGTGAGC AGTGGTTAAA AGACGTGCTA 720

GGATATCTGC TGGTACGGAG ACGACCAGCA AATTTTTCCT ACGACGTAAG AGTAGCTTGG 780

GTATATGACG TGATCGCTAC GCTCAAGCTG GTCATAAGAT TGTTTTTCAA CAAGGACACA 840

CCCGGGGGTA TTAAAGACTT AAAACCGTGT GTGCCTATAG AGTCATTCGA CCCCTTTCAC 900

GAGCTTTCGT CCTATTTCTC TAGGTTAAGT TACGAGATGA CGACAGGTAA AGGGGGAAAG 960

ATATGCCCGG AGATCGCCGA GAAGTTGGTG CGCCGTCTAA TGGAGGAAAA CTATAAGTTA 1020

AGATTGACCC CAGTGATGGC CTTAATAATT ATACTGGTAT ACTACTCCAT TTACGGCACA 1080

AACGCTACCA GGATTAAAAG ACGCCCGGAT TTCCTCAATG TGAGGATAAA GGGAAGAGTC 1140

GAGAAGGTTT CGTTACGGGG GGTAGAAGAT CGTGCCTTTA GAATATCAGA AAAGCGCGGG 1200

ATAAACGCTC AACGTGTATT ATGTAGGTAC TATAGCGATC TCACATGTCT GGCTAGGCGA 1260

CATTACGGCA TTCGCAGGAA CAATTGGAAG ACGCTGAGTT ATGTAGACGG GACGTTAGCG 1320

TATGACACGG CTGATTGTAT AACTTCTAAG GTGAGAAATA CGATCAACAC CGCAGATCAC 1380

GCTAGCATTA TACACTATAT CAAGACGAAC GAAAACCAGG TTACCGGAAC TACTCTACCA 1440

CACCAGCTTT AA 1452

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 483 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asp Lys Tyr Ile Tyr Val Thr Gly Ile Leu Asn Pro Asn Glu Ala
1 5 10 15

Arg Asp Glu Val Phe Ser Val Val Asn Lys Gly Tyr Ile Gly Pro Gly
20 25 30

Gly Arg Ser Phe Ser Asn Arg Gly Ser Lys Tyr Thr Val Val Trp Glu
35 40 45

Asn Ser Ala Ala Arg Ile Ser Gly Phe Thr Ser Thr Ser Gln Ser Thr
50 55 60

Ile Asp Ala Phe Ala Tyr Phe Leu Leu Lys Gly Gly Leu Thr Thr Thr
65 70 75 80

Leu Ser Asn Pro Ile Asn Cys Glu Asn Trp Val Arg Ser Ser Lys Asp
85 90 95

Leu Ser Ala Phe Phe Arg Thr Leu Ile Lys Gly Lys Ile Tyr Ala Ser
100 105 110

Arg Ser Val Asp Ser Asn Leu Pro Lys Lys Asp Arg Asp Asp Ile Met
115 120 125

Glu Ala Ser Arg Arg Leu Ser Pro Ser Asp Ala Ala Phe Cys Arg Ala
130 135 140

Val Ser Val Gln Val Gly Lys Tyr Val Asp Val Thr Gln Asn Leu Glu
145 150 155 160

Ser Thr Ile Val Pro Leu Arg Val Met Glu Ile Lys Lys Arg Arg Gly
165 170 175

Ser Ala His Val Ser Leu Pro Lys Val Val Ser Ala Tyr Val Asp Phe
180 185 190

Tyr Thr Asn Leu Gln Glu Leu Leu Ser Asp Glu Val Thr Arg Ala Arg
195 200 205

Thr Asp Thr Val Ser Ala Tyr Ala Thr Asp Ser Met Ala Phe Leu Val
210 215 220

Lys Met Leu Pro Leu Thr Ala Arg Glu Gln Trp Leu Lys Asp Val Leu
225 230 235 240

Gly Tyr Leu Leu Val Arg Arg Arg Pro Ala Asn Phe Ser Tyr Asp Val
245 250 255

Arg Val Ala Trp Val Tyr Asp Val Ile Ala Thr Leu Lys Leu Val Ile
260 265 270

Arg Leu Phe Phe Asn Lys Asp Thr Pro Gly Gly Ile Lys Asp Leu Lys
275 280 285

Pro Cys Val Pro Ile Glu Ser Phe Asp Pro Phe His Glu Leu Ser Ser
290 295 300

Tyr Phe Ser Arg Leu Ser Tyr Glu Met Thr Thr Gly Lys Gly Gly Lys
305 310 315 320

Ile Cys Pro Glu Ile Ala Glu Lys Leu Val Arg Arg Leu Met Glu Glu
325 330 335

Asn Tyr Lys Leu Arg Leu Thr Pro Val Met Ala Leu Ile Ile Ile Leu
340 345 350

Val Tyr Tyr Ser Ile Tyr Gly Thr Asn Ala Thr Arg Ile Lys Arg Arg
355 360 365

Pro Asp Phe Leu Asn Val Arg Ile Lys Gly Arg Val Glu Lys Val Ser
370 375 380

Leu Arg Gly Val Glu Asp Arg Ala Phe Arg Ile Ser Glu Lys Arg Gly
385 390 395 400

Ile Asn Ala Gln Arg Val Leu Cys Arg Tyr Tyr Ser Asp Leu Thr Cys
405 410 415

Leu Ala Arg Arg His Tyr Gly Ile Arg Arg Asn Asn Trp Lys Thr Leu
420 425 430

Ser Tyr Val Asp Gly Thr Leu Ala Tyr Asp Thr Ala Asp Cys Ile Thr
435 440 445

Ser Lys Val Arg Asn Thr Ile Asn Thr Ala Asp His Ala Ser Ile Ile
450 455 460

His Tyr Ile Lys Thr Asn Glu Asn Gln Val Thr Gly Thr Thr Leu Pro
465 470 475 480

His Gln Leu

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 942 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGGCATTTG AACTGAAATT AGGGCAGATA TATGAAGTCG TCCCCGAAAA TAATTTGAGA 60

GTTAGAGTGG GGGATGCGGC ACAAGGAAAA TTTAGTAAGG CGAGTTTCTT AAAGTACGTT 120

AAGGACGGGA CACAGGCGGA ATTAACGGGA ATCGCCGTAG TGCCCGAAAA ATACGTATTC 180

GCCACAGCAG CTTTGGCTAC AGCGGCGCAG GAGCCACCTA GGCAGCCACC AGCGCAAGTG 240

GCGGAACCAC AGGAAACCGA TATAGGGGTA GTGCCGGAAT CTGAGACTCT CACACCAAAT 300

AAGTTGGTTT TCGAGAAAGA TCCAGACAAG TTCTTGAAGA CTATGGGCAA GGGAATAGCT 360

TTGGACTTGG CGGGAGTTAC CCACAAACCG AAAGTTATTA ACGAGCCAGG GAAAGTATCA 420

GTAGAGGTGG CAATGAAGAT TAATGCCGCA TTGATGGAGC TGTGTAAGAA GGTTATGGGC 480

GCCGATGACG CAGCAACTAA GACAGAATTC TTCTTGTACG TGATGCAGAT TGCTTGCACG 540

TTCTTTACAT CGTCTTCGAC GGAGTTCAAA GAGTTTGACT ACATAGAAAC CGATGATGGA 600

AAGAAGATAT ATGCGGTGTG GGTATATGAT TGCATTAAAC AAGCTGCTGC TTCGACGGGT 660

TATGAAAACC CGGTAAGGCA GTATCTAGCG TACTTCACAC CAACCTTCAT CACGGCGACC 720

CTGAATGGTA AACTAGTGAT GAACGAGAAG GTTATGGCAC AGCATGGAGT ACCACCGAAA 780

TTCTTTCCGT ACACGATAGA CTGCGTTCGT CCGACGTACG ATCTGTTCAA CAACGACGCA 840

ATATTAGCAT GGAATTTAGC TAGACAGCAG GCGTTTAGAA ACAAGACGGT AACGGCCGAT 900

AACACCTTAC ACAACGTCTT CCAACTATTG CAAAAGAAGT AG 942

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 313 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Phe Glu Leu Lys Leu Gly Gln Ile Tyr Glu Val Val Pro Glu
1 5 10 15

Asn Asn Leu Arg Val Arg Val Gly Asp Ala Ala Gln Gly Lys Phe Ser
20 25 30

Lys Ala Ser Phe Leu Lys Tyr Val Lys Asp Gly Thr Gln Ala Glu Leu
35 40 45

Thr Gly Ile Ala Val Val Pro Glu Lys Tyr Val Phe Ala Thr Ala Ala
50 55 60

Leu Ala Thr Ala Ala Gln Glu Pro Pro Arg Gln Pro Pro Ala Gln Val
65 70 75 80

Ala Glu Pro Gln Glu Thr Asp Ile Gly Val Val Pro Glu Ser Glu Thr
85 90 95

Leu Thr Pro Asn Lys Leu Val Phe Glu Lys Asp Pro Asp Lys Phe Leu
100 105 110

Lys Thr Met Gly Lys Gly Ile Ala Leu Asp Leu Ala Gly Val Thr His
115 120 125

Lys Pro Lys Val Ile Asn Glu Pro Gly Lys Val Ser Val Glu Val Ala
130 135 140

Met Lys Ile Asn Ala Ala Leu Met Glu Leu Cys Lys Lys Val Met Gly
145 150 155 160

Ala Asp Asp Ala Ala Thr Lys Thr Glu Phe Phe Leu Tyr Val Met Gln
165 170 175

Ile Ala Cys Thr Phe Phe Thr Ser Ser Ser Thr Glu Phe Lys Glu Phe
180 185 190

Asp Tyr Ile Glu Thr Asp Asp Gly Lys Lys Ile Tyr Ala Val Trp Val
195 200 205

Tyr Asp Cys Ile Lys Gln Ala Ala Ala Ser Thr Gly Tyr Glu Asn Pro
210 215 220

Val Arg Gln Tyr Leu Ala Tyr Phe Thr Pro Thr Phe Ile Thr Ala Thr
225 230 235 240

Leu Asn Gly Lys Leu Val Met Asn Glu Lys Val Met Ala Gln His Gly
245 250 255

Val Pro Pro Lys Phe Phe Pro Tyr Thr Ile Asp Cys Val Arg Pro Thr
260 265 270

Tyr Asp Leu Phe Asn Asn Asp Ala Ile Leu Ala Trp Asn Leu Ala Arg
275 280 285

Gln Gln Ala Phe Arg Asn Lys Thr Val Thr Ala Asp Asn Thr Leu His
290 295 300

Asn Val Phe Gln Leu Leu Gln Lys Lys
305 310

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 156 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGTACAGTA GAGGGTCTTT CTTTAAGTCT CGGGTTACCC TTCCTACTCT TGTCGGAGCA 60

TACATGTGGG AGTTTGAACT CCCGTATCTT ACGGACAAGA GACACATCAG CTATAGCGCG 120

CCAAGTGTCG CGACTTTTAG CCTTGTGTCG AGGTAG 156

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Tyr Ser Arg Gly Ser Phe Phe Lys Ser Arg Val Thr Leu Pro Thr
1 5 10 15

Leu Val Gly Ala Tyr Met Trp Glu Phe Glu Leu Pro Tyr Leu Thr Asp
20 25 30

Lys Arg His Ile Ser Tyr Ser Ala Pro Ser Val Ala Thr Phe Ser Leu
35 40 45

Val Ser Arg
50

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 138 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGGATGATT TTAAACAGGC AATACTGTTG CTAGTAGTCG ATTTTGTCTT CGTGATAATT 60

CTGCTGCTGG TTCTTACGTT CGTCGTCCCG AGGTTACAGC AAAGCTCCAC CATTAATACA 120

GGTCTTAGGA CAGTGTGA 138

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Asp Asp Phe Lys Gln Ala Ile Leu Leu Val Val Asp Phe Val
1 5 10 15

Phe Val Ile Ile Leu Leu Leu Val Leu Thr Phe Val Val Pro Arg Leu
20 25 30

Gln Gln Ser Ser Thr Ile Asn Thr Gly Leu Arg Thr Val
35 40 45

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1434 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATGGGAGCTT ATACACATGT AGACTTTCAT GAGTCGCGGT TGCTGAAAGA CAAACAAGAC 60

TATCTTTCTT TCAAGTCAGC GGATGAAGCT CCTCCTGATC CTCCCGGATA CGTTCGCCCA 120

GATAGTTATG TGAGGGCTTA TTTGATACAA AGAGCAGACT TTCCCAATAC TCAAAGCTTA 180

TCAGTTACGT TATCGATAGC CAGTAATAAG TTAGCTTCAG GTCTTATGGG AAGCGACGCA 240

GTATCATCGT CGTTTATGCT GATGAACGAC GTGGGAGATT ACTTCGAGTG CGGCGTGTGT 300

CACAACAAAC CCTACTTAGG ACGGGAAGTT ATCTTCTGTA GGAAATACAT AGGTGGGAGA 360

GGAGTGGAGA TCACCACTGG TAAGAACTAC ACGTCGAACA ATTGGAACGA GGCGTCGTAC 420

GTAATACAAG TGAACGTAGT CGATGGGTTA GCACAGACCA CTGTTAATTC TACTTATACG 480

CAAACGGACG TTAGTGGTCT ACCCAAAAAT TGGACGCGTA TCTACAAAAT AACAAAGATA 540

GTGTCCGTAG ATCAGAACCT CTACCCTGGT TGTTTCTCAG ACTCGAAACT GGGTGTAATG 600

CGTATAAGGT CACTGTTAGT TTCCCCAGTG CGCATCTTCT TTAGGGATAT CTTATTGAAA 660

CCTTTGAAGA AATCGTTCAA CGCAAGAATC GAGGATGTGC TGAATATTGA CGACACGTCG 720

TTGTTAGTAC CGAGTCCTGT CGTACCAGAG TCTACGGGAG GTGTAGGTCC ATCAGAGCAG 780

CTGGATGTAG TGGCTTTAAC GTCCGACGTA ACGGAATTGA TCAACACTAG GGGGCAAGGT 840

AAGATATGTT TTCCAGACTC AGTGTTATCG ATCAATGAAG CGGATATCTA CGATGAGCGG 900

TATTTGCCGA TAACGGAAGC TCTACAGATA AACGCAAGAC TACGCAGACT CGTTCTTTCG 960

AAAGGCGGGA GTCAAACACC ACGAGATATG GGGAATATGA TAGTGGCCAT GATACAACTT 1020

TTCGTACTCT ACTCTACTGT AAAGAATATA AGCGTCAAAG ACGGGTATAG GGTGGAGACC 1080

GAATTAGGTC AAAAGAGAGT CTACTTAAGT TATTCGGAAG TAAGGGAAGC TATATTAGGA 1140

GGGAAATACG GTGCGTCTCC AACCAACACT GTGCGATCCT TCATGAGGTA TTTTGCTCAC 1200

ACCACTATTA CTCTACTTAT AGAGAAGAAA ATTCAGCCAG CGTGTACTGC CCTAGCTAAG 1260

CACGGCGTCC CGAAGAGGTT CACTCCGTAC TGCTTCGACT TCGCACTACT GGATAACAGA 1320

TATTACCCGG CGGACGTGTT GAAGGCTAAC GCAATGGCTT GCGCTATAGC GATTAAATCA 1380

GCTAATTTAA GGCGTAAAGG TTCGGAGACG TATAACATCT TAGAAAGCAT TTGA 1434

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 477 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Gly Ala Tyr Thr His Val Asp Phe His Glu Ser Arg Leu Leu Lys
1 5 10 15

Asp Lys Gln Asp Tyr Leu Ser Phe Lys Ser Ala Asp Glu Ala Pro Pro
20 25 30

Asp Pro Pro Gly Tyr Val Arg Pro Asp Ser Tyr Val Arg Ala Tyr Leu
35 40 45

Ile Gln Arg Ala Asp Phe Pro Asn Thr Gln Ser Leu Ser Val Thr Leu
50 55 60

Ser Ile Ala Ser Asn Lys Leu Ala Ser Gly Leu Met Gly Ser Asp Ala
65 70 75 80

Val Ser Ser Ser Phe Met Leu Met Asn Asp Val Gly Asp Tyr Phe Glu
85 90 95

Cys Gly Val Cys His Asn Lys Pro Tyr Leu Gly Arg Glu Val Ile Phe
100 105 110

Cys Arg Lys Tyr Ile Gly Gly Arg Gly Val Glu Ile Thr Thr Gly Lys
115 120 125

Asn Tyr Thr Ser Asn Asn Trp Asn Glu Ala Ser Tyr Val Ile Gln Val
130 135 140

Asn Val Val Asp Gly Leu Ala Gln Thr Thr Val Asn Ser Thr Tyr Thr
145 150 155 160

Gln Thr Asp Val Ser Gly Leu Pro Lys Asn Trp Thr Arg Ile Tyr Lys
165 170 175

Ile Thr Lys Ile Val Ser Val Asp Gln Asn Leu Tyr Pro Gly Cys Phe
180 185 190

Ser Asp Ser Lys Leu Gly Val Met Arg Ile Arg Ser Leu Leu Val Ser
195 200 205

Pro Val Arg Ile Phe Phe Arg Asp Ile Leu Leu Lys Pro Leu Lys Lys
210 215 220

Ser Phe Asn Ala Arg Ile Glu Asp Val Leu Asn Ile Asp Asp Thr Ser
225 230 235 240

Leu Leu Val Pro Ser Pro Val Val Pro Glu Ser Thr Gly Gly Val Gly
245 250 255

Pro Ser Glu Gln Leu Asp Val Val Ala Leu Thr Ser Asp Val Thr Glu
260 265 270

Leu Ile Asn Thr Arg Gly Gln Gly Lys Ile Cys Phe Pro Asp Ser Val
275 280 285

Leu Ser Ile Asn Glu Ala Asp Ile Tyr Asp Glu Arg Tyr Leu Pro Ile
290 295 300

Thr Glu Ala Leu Gln Ile Asn Ala Arg Leu Arg Arg Leu Val Leu Ser
305 310 315 320

Lys Gly Gly Ser Gln Thr Pro Arg Asp Met Gly Asn Met Ile Val Ala
325 330 335

Met Ile Gln Leu Phe Val Leu Tyr Ser Thr Val Lys Asn Ile Ser Val
340 345 350

Lys Asp Gly Tyr Arg Val Glu Thr Glu Leu Gly Gln Lys Arg Val Tyr
355 360 365

Leu Ser Tyr Ser Glu Val Arg Glu Ala Ile Leu Gly Gly Lys Tyr Gly
370 375 380

Ala Ser Pro Thr Asn Thr Val Arg Ser Phe Met Arg Tyr Phe Ala His
385 390 395 400

Thr Thr Ile Thr Leu Leu Ile Glu Lys Lys Ile Gln Pro Ala Cys Thr
405 410 415

Ala Leu Ala Lys His Gly Val Pro Lys Arg Phe Thr Pro Tyr Cys Phe
420 425 430

Asp Phe Ala Leu Leu Asp Asn Arg Tyr Tyr Pro Ala Asp Val Leu Lys
435 440 445

Ala Asn Ala Met Ala Cys Ala Ile Ala Ile Lys Ser Ala Asn Leu Arg
450 455 460

Arg Lys Gly Ser Glu Thr Tyr Asn Ile Leu Glu Ser Ile
465 470 475

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 558 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGGAATTCA GACCAGTTTT AATTACAGTT CGCCGTGATC CCGGCGTAAA CACTGGTAGT 60

TTGAAAGTGA TAGCTTATGA CTTACACTAC GACAATATAT TCGATAACTG CGCGGTAAAG 120

TCGTTTCGAG ACACCGACAC TGGATTCACT GTTATGAAAG AATACTCGAC GAATTCAGCG 180

TTCATACTAA GTCCTTATAA ACTGTTTTCC GCGGTCTTTA ATAAGGAAGG TGAGATGATA 240

AGTAACGATG TAGGATCGAG TTTCAGGGTT TACAATATCT TTTCGCAAAT GTGTAAAGAT 300

ATCAACGAGA TCAGCGAGAT ACAACGCGCC GGTTACCTAG AAACATATTT AGGAGACGGG 360

CAGGCTGACA CTGATATATT TTTTGATGTC TTAACCAACA ACAAAGCAAA GGTAAGGTGG 420

TTAGTTAATA AAGACCATAG CGCGTGGTGT GGGATATTGA ATGATTTGAA GTGGGAAGAG 480

AGCAACAAGG AGAAATTTAA GGGGAGAGAC ATACTAGATA CTTACGTTTT ATCGTCTGAT 540

TATCCAGGGT TTAAATGA 558

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 185 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Glu Phe Arg Pro Val Leu Ile Thr Val Arg Arg Asp Pro Gly Val
1 5 10 15

Asn Thr Gly Ser Leu Lys Val Ile Ala Tyr Asp Leu His Tyr Asp Asn
20 25 30

Ile Phe Asp Asn Cys Ala Val Lys Ser Phe Arg Asp Thr Asp Thr Gly
35 40 45

Phe Thr Val Met Lys Glu Tyr Ser Thr Asn Ser Ala Phe Ile Leu Ser
50 55 60

Pro Tyr Lys Leu Phe Ser Ala Val Phe Asn Lys Glu Gly Glu Met Ile
65 70 75 80

Ser Asn Asp Val Gly Ser Ser Phe Arg Val Tyr Asn Ile Phe Ser Gln
85 90 95

Met Cys Lys Asp Ile Asn Glu Ile Ser Glu Ile Gln Arg Ala Gly Tyr
100 105 110

Leu Glu Thr Tyr Leu Gly Asp Gly Gln Ala Asp Thr Asp Ile Phe Phe
115 120 125

Asp Val Leu Thr Asn Asn Lys Ala Lys Val Arg Trp Leu Val Asn Lys
130 135 140

Asp His Ser Ala Trp Cys Gly Ile Leu Asn Asp Leu Lys Trp Glu Glu
145 150 155 160

Ser Asn Lys Glu Lys Phe Lys Gly Arg Asp Ile Leu Asp Thr Tyr Val
165 170 175

Leu Ser Ser Asp Tyr Pro Gly Phe Lys
180 185

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 534 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATGAAGTTGC TTTCGCTCCG CTATCTTATC TTAAGGTTGT CAAAGTCGCT TAGAACGAAC 60

GATCACTTGG TTTTAATACT TATAAAGGAG GCGCTTATAA ACTATTACAA CGCCTCTTTC 120

ACCGATGAGG GTGCCGTATT AAGAGACTCT CGCGAAAGTA TAGAGAATTT TCTCGTAGCC 180

AGGTGCGGTT CGCAAAATTC CTGCCGAGTC ATGAAGGCTT TGATCACTAA CACAGTCTGT 240

AAGATGTCGA TAGAAACAGC CAGAAGTTTT ATCGGAGACT TAATACTCGT CGCCGACTCC 300

TCTGTTTCAG CGTTGGAAGA AGCGAAATCA ATTAAAGATA ATTTCCGCTT AAGAAAAAGG 360

AGAGGCAAGT ATTATTATAG TGGTGATTGT GGATCCGACG TTGCGAAAGT TAAGTATATT 420

TTGTCTGGGG AGAATCGAGG ATTGGGGTGC GTAGATTCCT TGAAGCTAGT TTGCGTAGGT 480

AGACAAGGAG GTGGAAACGT ACTACAGCAC CTACTAATCT CATCTCTGGG TTAA 534

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Lys Leu Leu Ser Leu Arg Tyr Leu Ile Leu Arg Leu Ser Lys Ser
1 5 10 15

Leu Arg Thr Asn Asp His Leu Val Leu Ile Leu Ile Lys Glu Ala Leu
20 25 30

Ile Asn Tyr Tyr Asn Ala Ser Phe Thr Asp Glu Gly Ala Val Leu Arg
35 40 45

Asp Ser Arg Glu Ser Ile Glu Asn Phe Leu Val Ala Arg Cys Gly Ser
50 55 60

Gln Asn Ser Cys Arg Val Met Lys Ala Leu Ile Thr Asn Thr Val Cys
65 70 75 80

Lys Met Ser Ile Glu Thr Ala Arg Ser Phe Ile Gly Asp Leu Ile Leu
85 90 95

Val Ala Asp Ser Ser Val Ser Ala Leu Glu Glu Ala Lys Ser Ile Lys
100 105 110

Asp Asn Phe Arg Leu Arg Lys Arg Arg Gly Lys Tyr Tyr Tyr Ser Gly
115 120 125

Asp Cys Gly Ser Asp Val Ala Lys Val Lys Tyr Ile Leu Ser Gly Glu
130 135 140

Asn Arg Gly Leu Gly Cys Val Asp Ser Leu Lys Leu Val Cys Val Gly
145 150 155 160

Arg Gln Gly Gly Gly Asn Val Leu Gln His Leu Leu Ile Ser Ser Leu
165 170 175

Gly

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 540 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATGGACCTAT CGTTTATTAT TGTGCAGATC CTTTCCGCCT CGTACAATAA TGACGTGACA 60

GCACTTTACA CTTTGATTAA CGCGTATAAT AGCGTTGATG ATACGACGCG CTGGGCAGCG 120

ATAAACGATC CGCAAGCTGA GGTTAACGTC GTGAAGGCTT ACGTAGCTAC TACAGCGACG 180

ACTGAGCTGC ATAGAACAAT TCTCATTGAC AGTATAGACT CCGCCTTCGC TTATGACCAA 240

GTGGGGTGTT TGGTGGGCAT AGCTAGAGGT TTGCTTAGAC ATTCGGAAGA TGTTCTGGAG 300

GTCATCAAGT CGATGGAGTT ATTCGAAGTG TGTCGTGGAA AGAGGGGAAG CAAAAGATAT 360

CTTGGATACT TAAGTGATCA ATGCACTAAC AAATACATGA TGCTAACTCA GGCCGGACTG 420

GCCGCAGTTG AAGGAGCAGA CATACTACGA ACGAATCATC TAGTCAGTGG TAATAAGTTC 480

TCTCCAAATT TCGGGATCGC TAGGATGTTG CTCTTGACGC TTTGTTGCGG AGCACTATAA 540

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 179 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Asp Leu Ser Phe Ile Ile Val Gln Ile Leu Ser Ala Ser Tyr Asn
1 5 10 15

Asn Asp Val Thr Ala Leu Tyr Thr Leu Ile Asn Ala Tyr Asn Ser Val
20 25 30

Asp Asp Thr Thr Arg Trp Ala Ala Ile Asn Asp Pro Gln Ala Glu Val
35 40 45

Asn Val Val Lys Ala Tyr Val Ala Thr Thr Ala Thr Thr Glu Leu His
50 55 60

Arg Thr Ile Leu Ile Asp Ser Ile Asp Ser Ala Phe Ala Tyr Asp Gln
65 70 75 80

Val Gly Cys Leu Val Gly Ile Ala Arg Gly Leu Leu Arg His Ser Glu
85 90 95

Asp Val Leu Glu Val Ile Lys Ser Met Glu Leu Phe Glu Val Cys Arg
100 105 110

Gly Lys Arg Gly Ser Lys Arg Tyr Leu Gly Tyr Leu Ser Asp Gln Cys
115 120 125

Thr Asn Lys Tyr Met Met Leu Thr Gln Ala Gly Leu Ala Ala Val Glu
130 135 140

Gly Ala Asp Ile Leu Arg Thr Asn His Leu Val Ser Gly Asn Lys Phe
145 150 155 160

Ser Pro Asn Phe Gly Ile Ala Arg Met Leu Leu Leu Thr Leu Cys Cys
165 170 175

Gly Ala Leu

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATGAGGCACT TAGAAAAACC CATCAGAGTA GCGGTACACT ATTGCGTCGT GCGAAGTGAC 60

GTTTGTGACG GGTGGGATGT ATTTATAGGC GTAACGTTAA TCGGTATGTT TATTAGTTAC 120

TATTTATATG CTCTAATTAG CATATGTAGA AAAGGAGAAG GTTTAACAAC CAGTAATGGG 180

TAA 183

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Arg His Leu Glu Lys Pro Ile Arg Val Ala Val His Tyr Cys Val
1 5 10 15

Val Arg Ser Asp Val Cys Asp Gly Trp Asp Val Phe Ile Gly Val Thr
20 25 30

Leu Ile Gly Met Phe Ile Ser Tyr Tyr Leu Tyr Ala Leu Ile Ser Ile
35 40 45

Cys Arg Lys Gly Glu Gly Leu Thr Thr Ser Asn Gly
50 55 60

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide"

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..24
(D) OTHER INFORMATION: /product= "Oligonucleotide"
/note= "N is inosine at sites in this sequence."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGNGGNGGNA CNTTYGAYGT NTCN 24

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

UGAGUGAACG CGAUG 15

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATAAGCATTC GGGATGGACC 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ATTAACTTGA CGGATGGCAC GC 22

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TACTTATCTA GAACCATGGA AGCGAGTCGA CGACTA 36

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCTTGAGGAT CCATGGAGAA ACATCGTCGC ATACTA 36

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

ACTATTTCTA GAACCATGGC ATTTGAACTG AAATT 35

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide."

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TTCTGAGGAT CCATGGTATA AGCTCCCATG AATTAT 36

TABLE 1

Particle length (nm)	Coat protein Mr ($X10^3$)	Reference
1,400-2,200	39	Gugerli (1984)
1,400-1,800	26	Gugerli (1984)
1,400-2,200	43	Zimmermann (1990)
1,400-2,200	36	Zee (1987)
1,400-2,200	36	Hu (1990)
1,400-2,200	36	Zimmermann (1990)
		Gugerli (1993)

TABLE 2

Nucleotide and Deduced Amino Acid Sequences for GLRaV-3 Coat Protein.

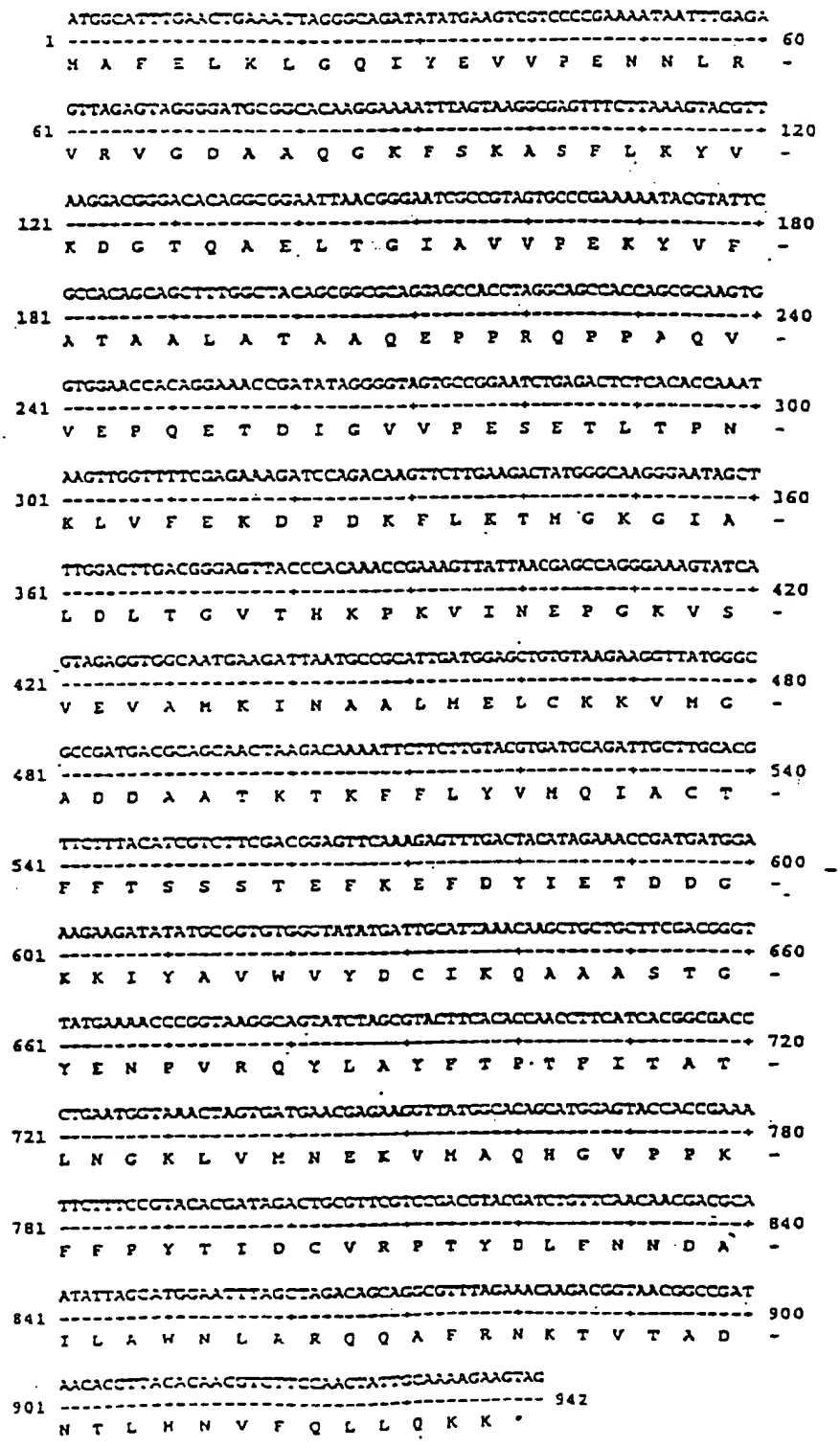


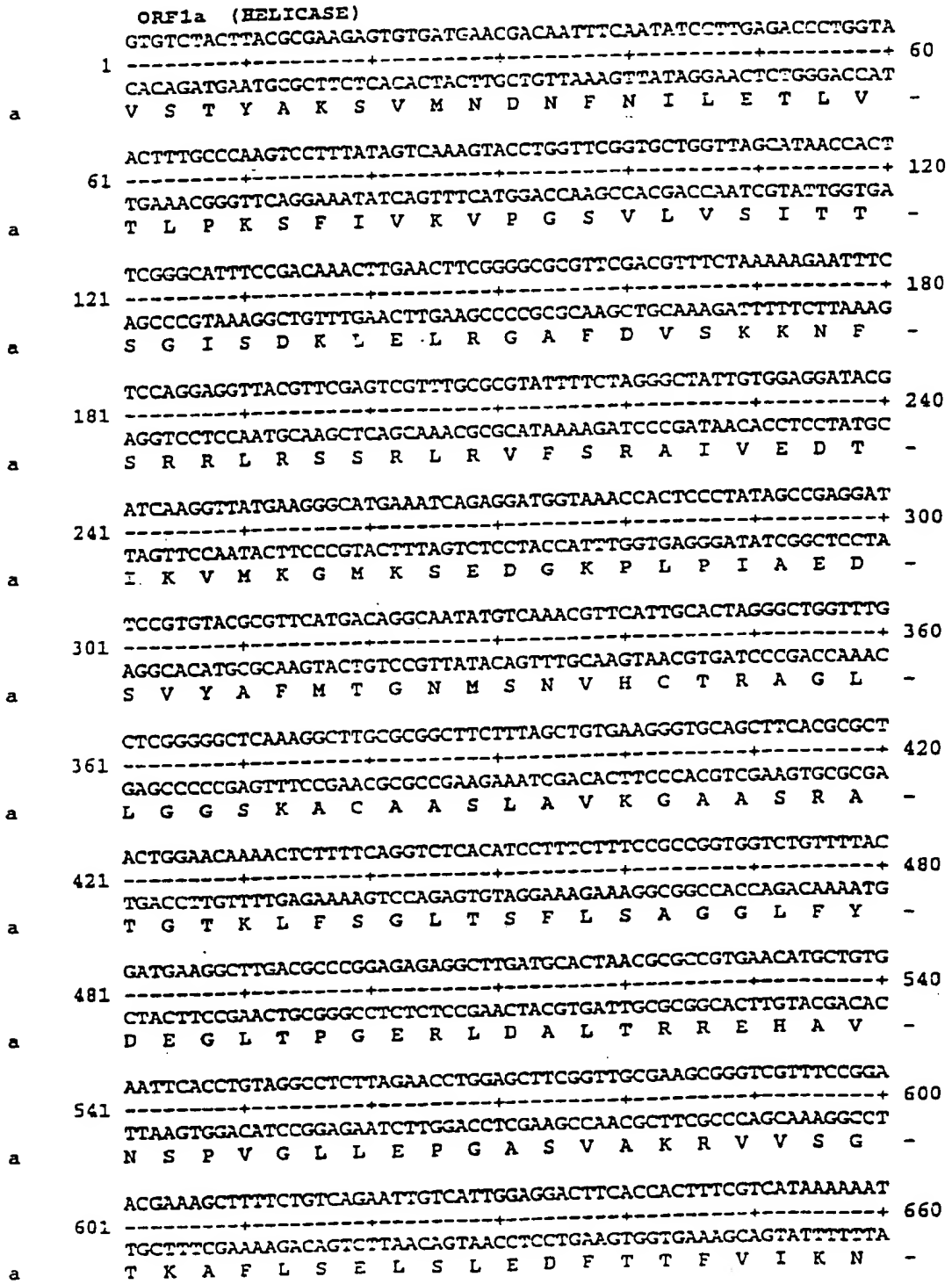
TABLE 3

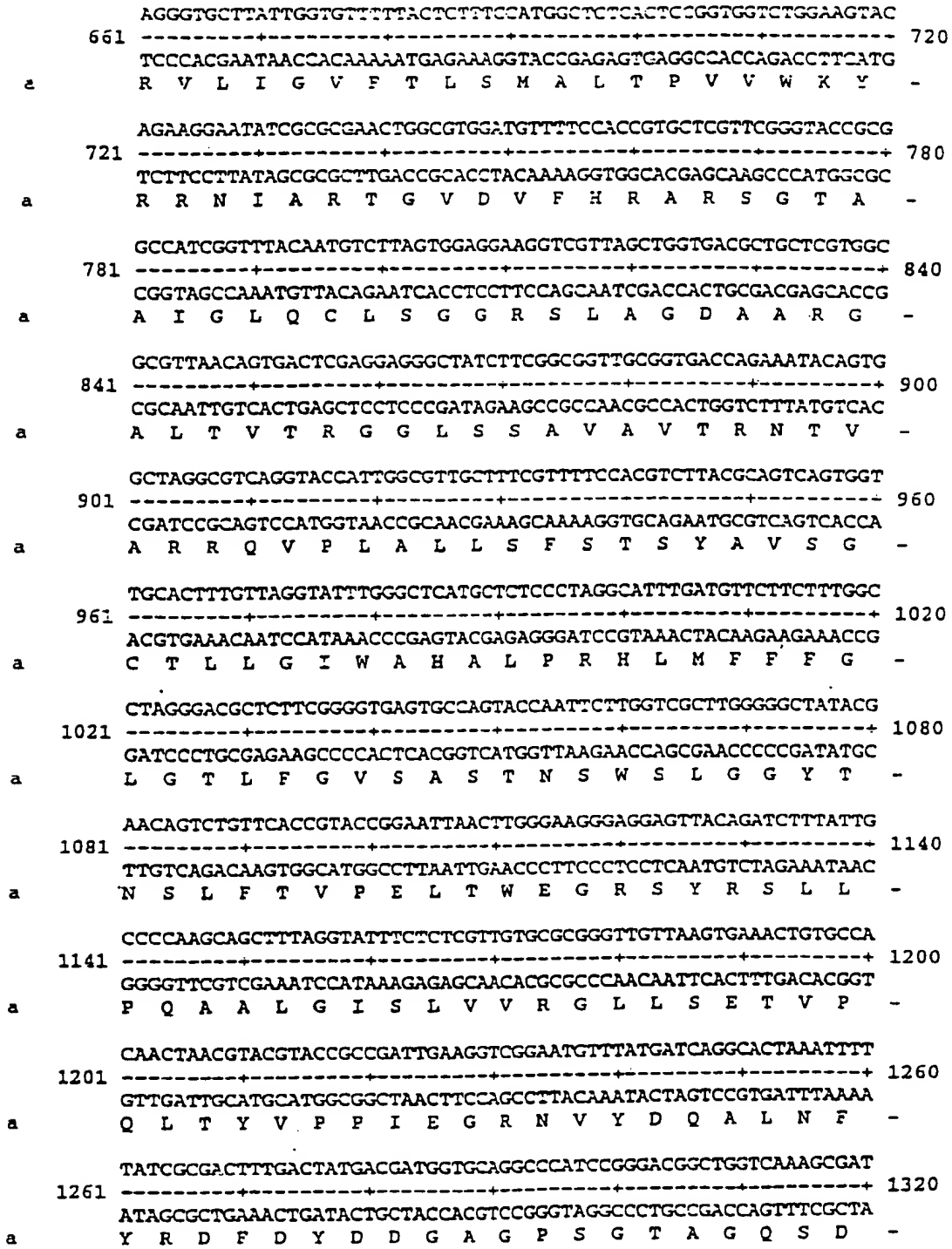
Comparison of Coat Protein Sequences of GLRaV-3 with BTV, CTV and LIYV.

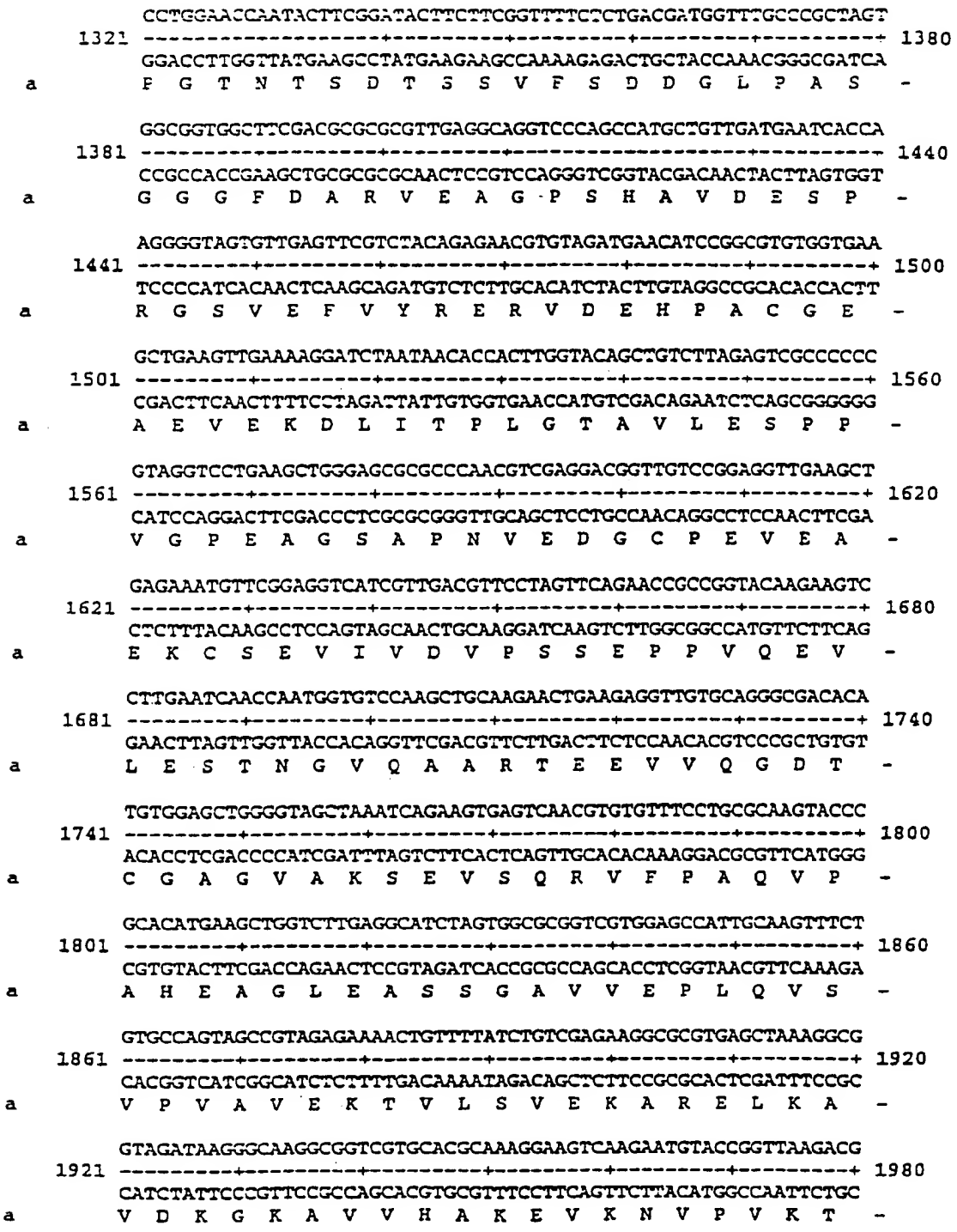
```
              1                                                    50
     BYV_CP   .......... .......... .......... .......... ..........
     CTV_CP   .......... .......... .......... .......... ..........
    LIYV_CP   .......... .......... .......... .......... ..........
  GLRaV3_CP   MAFELKLGQI YEVVPENNLR VRVGDAAQGK FSKASFLKYV KDGTQAELTG

  CONSENSUS   ---------- ---------- ---------- ---------- ----------

              51                                                  100
     BYV_CP   .......... .......... .......... .......... ..........
     CTV_CP   .......... .......... .......... .......... MDDETKKLKN
    LIYV_CP   .......... ....MDTDGD NDVFGSGNDT RNNDDKKKEE MKQNISDNSQ
  GLRaV3_CP   IAVVPEKYVF ATAALATAAQ EPPRQPPAQV VEPQETDMGV VPESETLTPN

  CONSENSUS   ---------- ---------- ---------- ---------- ----------

              101                                                 150
     BYV_CP   .....MGSAE PISAIA..TF ENVSL.AD.Q TCLHGEDCDK LRK......N
     CTV_CP   KNKETKEGDD VVAAES..SF GSVNLHID.P TLITMNDVRQ LSTQQNAALN
    LIYV_CP   IISTRDHEAD IIGSISKEDL SKIVVRVDRH DALSANDVQS FR...EAMIN
  GLRaV3_CP   KLVFEKDPDK FLKTMGKGIA LDLTGVTHKP KVI..NEPGK VSVEVAMKIN

  CONSENSUS   ---------d -i-------f --v----d-- -----nd--- l--------N

              151                                                 200
     BYV_CP   FEECLKLKG. ...VPEDNLG IALGLCLYSC AT.IGTSNKV NVQPTS.TFI
     CTV_CP   RDLFLALKGK YPNLPDKDKD FHIAMMLYRL AV.KSSSLQS DDDTTGITYT
    LIYV_CP   ...FMRDKDP NRNQPSDKLI IAMEVGVYQH VDILGTSAKL G.NANNLEFT
  GLRaV3_CP   .AALMELCKK VMGADDAATK TKFFLYVMQI ACTFFTS..S STEFKEFDYI

  CONSENSUS   ---f--lk-- ----pd---- ----l--y-- a----cS--- ----------

              201                                                 250
     BYV_CP   KASFGGGKEL YLTHGELNSF LGSQKLLEGK PNKLRCFCRT FQKDYISLRK
     CTV_CP   R....EGVEV DLSDKLWTDI VYNSKGIGNR TNALRVWGRT NDALYLAFCR
    LIYV_CP   IAYDQETRTY KVAD..FVNY MQSR..MRNS PNVVRQYARA MEKTINNIRS
  GLRaV3_CP   ET..DDGKKI Y..AVWVYDC IKQAAASTGY ENPVRQYLAY FTPTFITATL

  CONSENSUS   -----eg--- ---------- ---------- -N--R-y-r- ----y-----

              251                                                 300
     BYV_CP   EYRGKLPPIA RANRHGLPAE DHYLAADP.I STSTELTDLQ QSRLLLAREN
     CTV_CP   QNR.NLSYGG RPLDAGIPAG YHYLCADP.L .TGAGLTDLE CAVYIQAKEQ
    LIYV_CP   AGIIN.SNGV LAAKHGVLAS YRNSYSDPAV GFGNDTTDAQ LTSLMLARKQ
  GLRaV3_CP   NGKLVMNEKV MA.QHGVPPK FFPYTIDCVR PTYDLFNNDA ILAWNLARQQ

  CONSENSUS   -----l---- -a--hGvpa- y-----Df-- -t---ltd-- -----lAr-q

              301                         328
     BYV_CP   ATH.TEFSSE SPVTSLKQLG RGLGTGR*
     CTV_CP   LLK.KRGADE VVVTNVRQLG K.FNTR*.
    LIYV_CP   ALC.KGEGGS VEHTNTMQLA NLKHPC*.
  GLRaV3_CP   AFRNKTVTAD NTLHNVFQLL QKK*....

  CONSENSUS   al--k----e ----n--QL- --------
```

TABLE 4

Partial GLRaV-3 Nucleotide Sequence and Encoded Proteins.




AGGGTGCTTATTGGTGTTTTTACTCTTTCCATGGCTCTCACTCCGGTGGTCTGGAAGTAC
661 720
TCCCACGAATAACCACAAAAATGAGAAAGGTACCGAGAGTGAGGCCACCAGACCTTCATG
R V L I G V F T L S M A L T P V V W K Y
AGAAGGAATATCGCGCGAACTGGCGTGGATGTTTTCCACCGTGCTCGTTCGGGTACCGCG
721 780
TCTTCCTTATAGCGCGCTTGACCGCACCTACAAAAGGTGGCACGAGCAAGCCCATGGCGC
R R N I A R T G V D V F H R A R S G T A
GCCATCGGTTTACAATGTCTTAGTGGAGGAAGGTCGTTAGCTGGTGACGCTGCTCGTGGC
781 840
CGGTAGCCAAATGTTACAGAATCACCTCCTTCCAGCAATCGACCACTGCGACGAGCACCG
A I G L Q C L S G G R S L A G D A A R G
GCGTTAACAGTGACTCGAGGAGGGCTATCTTCGGCGGTTGCGGTGACCAGAAATACAGTG
841 900
CGCAATTGTCACTGAGCTCCTCCCGATAGAAGCCGCCAACGCCACTGGTCTTTATGTCAC
A L T V T R G G L S S A V A V T R N T V
GCTAGGCGTCAGGTACCATTGGCGTTGCTTTCGTTTTCCACGTCTTACGCAGTCAGTGGT
901 960
CGATCCGCAGTCCATGGTAACCGCAACGAAAGCAAAAGGTGCAGAATGCGTCAGTCACCA
A R R Q V P L A L L S F S T S Y A V S G
TGCACTTTGTTAGGTATTTGGGCTCATGCTCTCCCTAGGCATTTGATGTTCTTCTTTGGC
961 1020
ACGTGAAACAATCCATAAACCCGAGTACGAGAGGGATCCGTAAACTACAAGAAGAAACCG
C T L L G I W A H A L P R H L M F F F G
CTAGGGACGCTCTTCGGGGTGAGTGCCAGTACCAATTCTTGGTCGCTTGGGGGCTATACG
1021 1080
GATCCCTGCGAGAAGCCCCACTCACGGTCATGGTTAAGAACCAGCGAACCCCCGATATGC
L G T L F G V S A S T N S W S L G G Y T
AACAGTCTGTTCACCGTACCGGAATTAACTTGGGAAGGGAGGAGTTACAGATCTTTATTG
1081 1140
TTGTCAGACAAGTGGCATGGCCTTAATTGAACCCTTCCCTCCTCAATGTCTAGAAATAAC
N S L F T V P E L T W E G R S Y R S L L
CCCCAAGCAGCTTTAGGTATTTCTCTCGTTGTGCGCGGGTTGTTAAGTGAAACTGTGCCA
1141 1200
GGGGTTCGTCGAAATCCATAAAGAGAGCAACACGCGCCCAACAATTCACTTTGACACGGT
P Q A A L G I S L V V R G L L S E T V P
CAACTAACGTACGTACCGCCGATTGAAGGTCGGAATGTTTATGATCAGGCACTAAATTTT
1201 1260
GTTGATTGCATGCATGGCGGCTAACTTCCAGCCTTACAAATACTAGTCCGTGATTTAAAA
Q L T Y V P P I E G R N V Y D Q A L N F
TATCGCGACTTTGACTATGACGATGGTGCAGGCCCATCCGGGACGGCTGGTCAAAGCGAT
1261 1320
ATAGCGCTGAAACTGATACTGCTACCACGTCCGGGTAGGCCCTGCCGACCAGTTTCGCTA
Y R D F D Y D D G A G P S G T A G Q S D


CCTGGAACCAATACTTCGGATACTTCTTCGGTTTTCCCTGACGATGGTTTGCCCGCTAGT
1321 ---------+---------+---------+---------+---------+---------+ 1380
GGACCTTGGTTATGAAGCCTATGAAGAAGCCAAAAGAGACTGCTACCAAACGGGCGATCA
a P G T N T S D T S S V F S D D G L P A S -
GGCGGTGGCTTCGACGCGCGCGTTGAGGCAGGTCCCAGCCATGCTGTTGATGAATCACCA
1381 ---------+---------+---------+---------+---------+---------+ 1440
CCGCCACCGAAGCTGCGCGCGCAACTCCGTCCAGGGTCGGTACGACAACTACTTAGTGGT
a G G G F D A R V E A G P S H A V D E S P -
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1441 ---------+---------+---------+---------+---------+---------+ 1500
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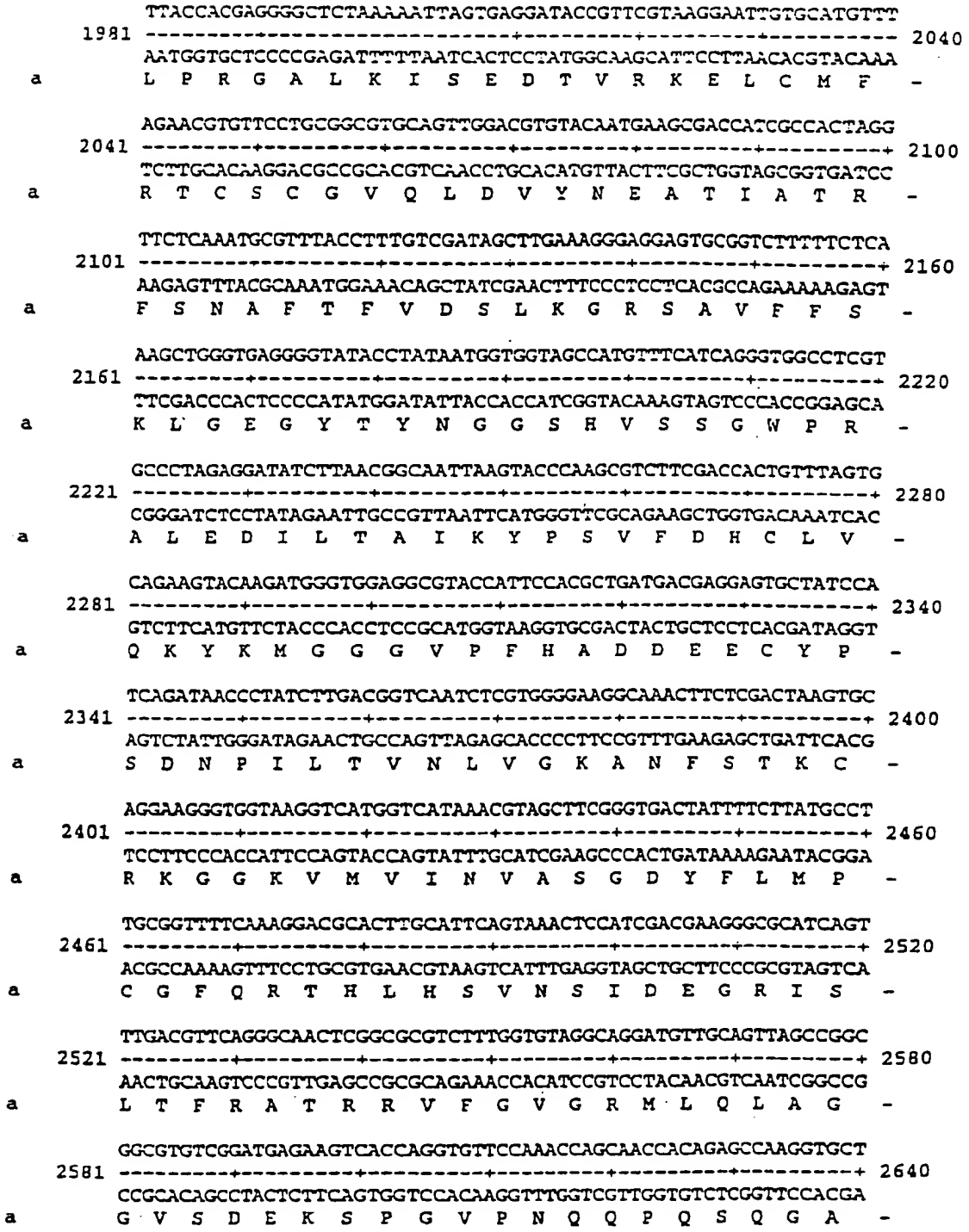
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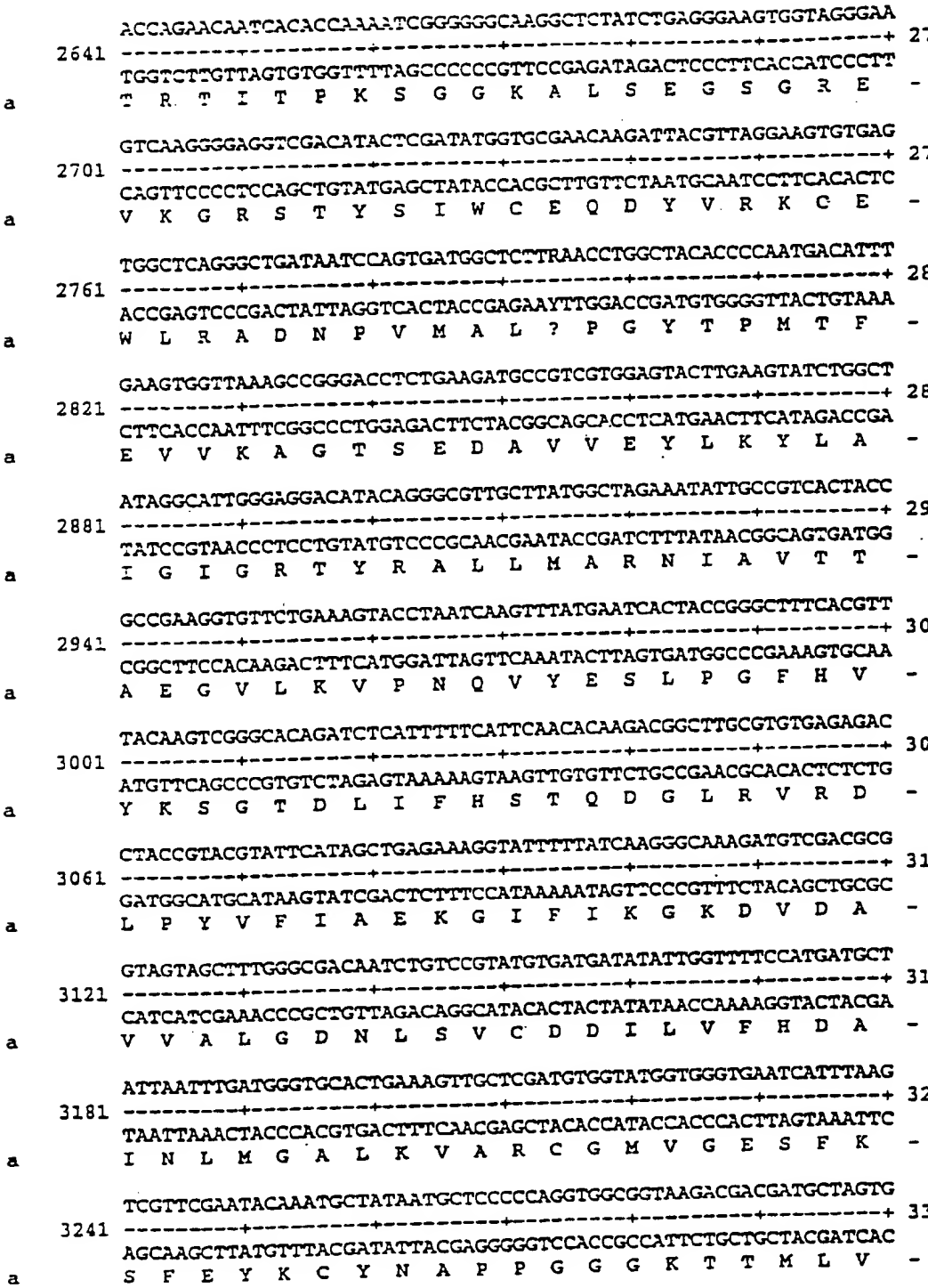
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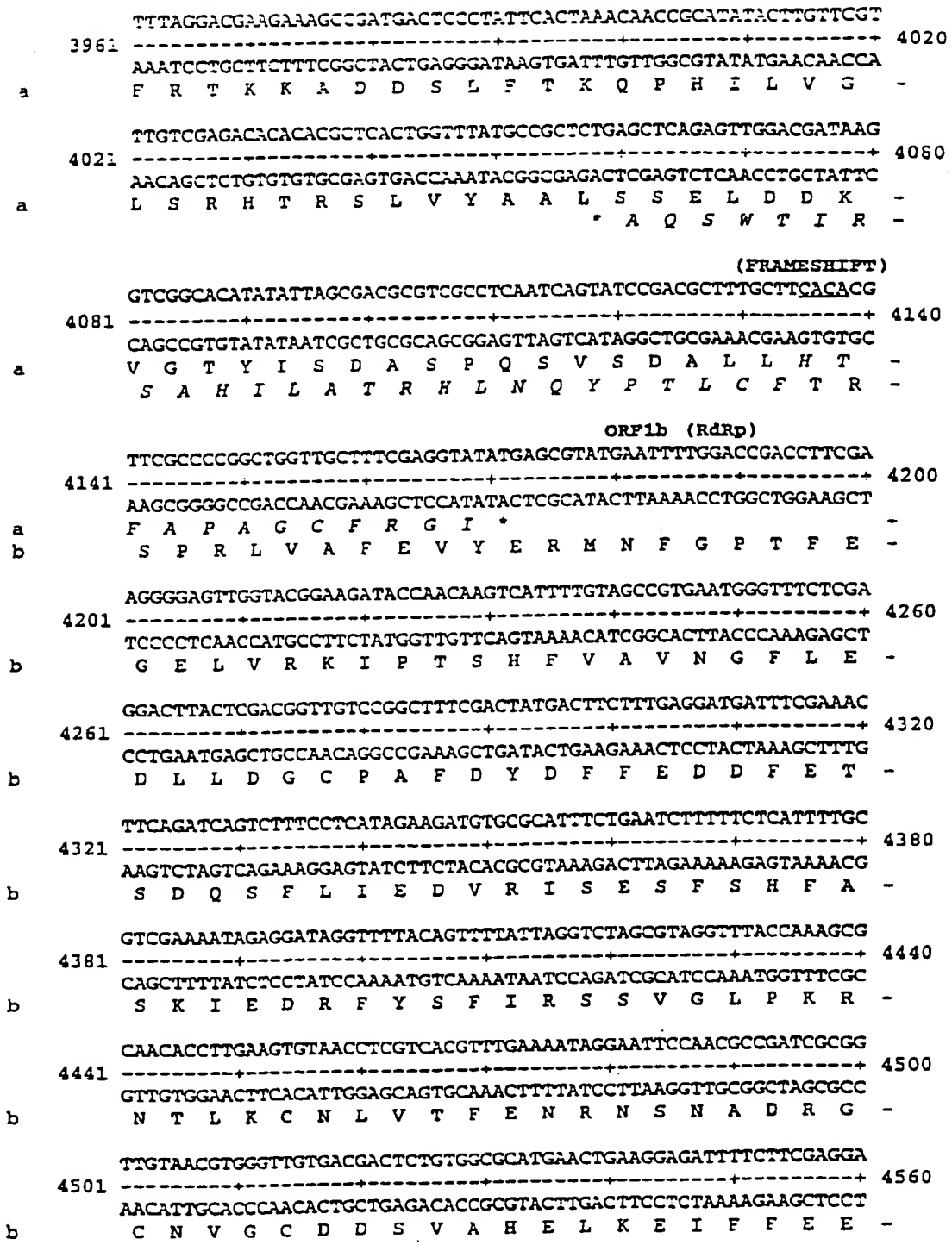
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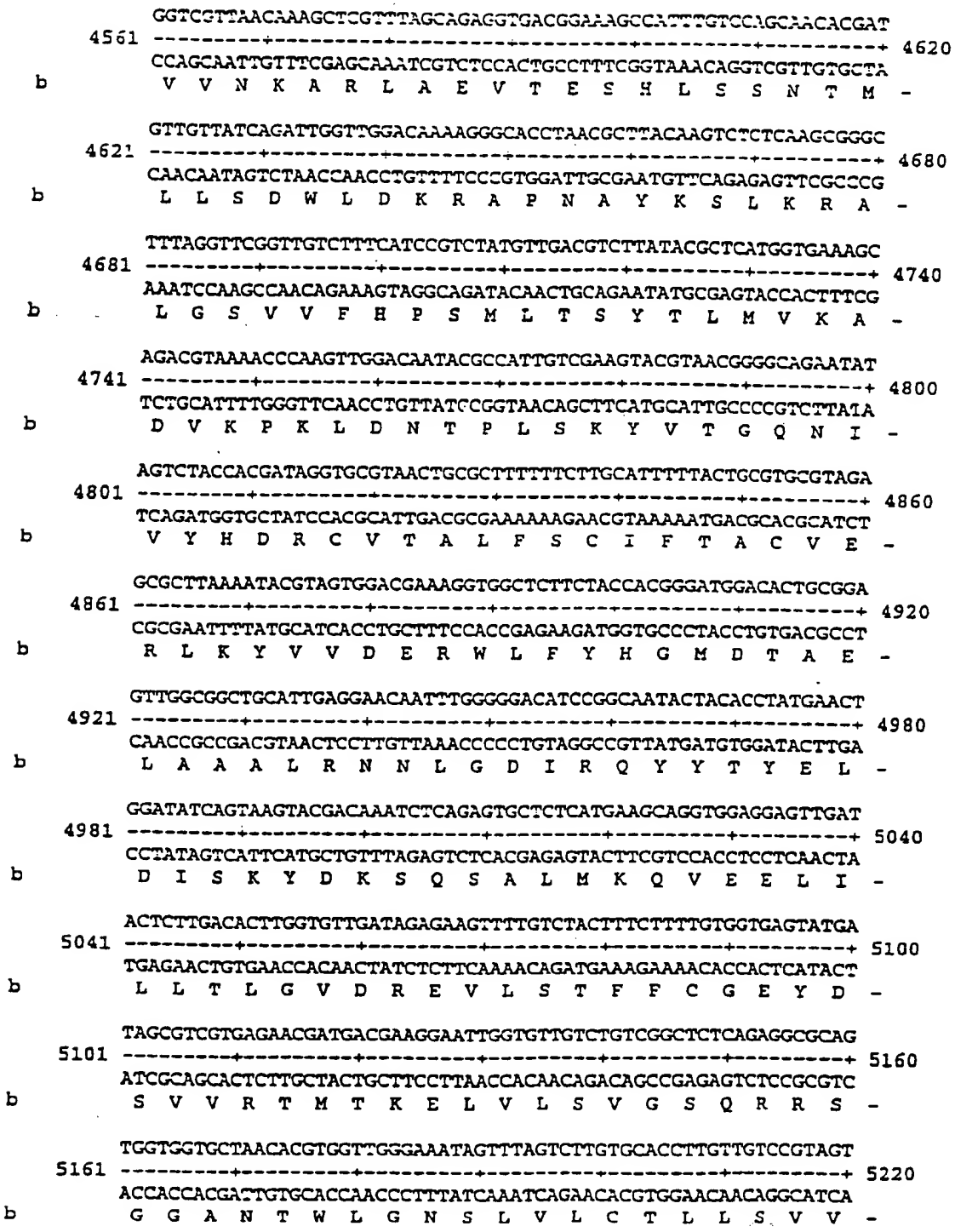
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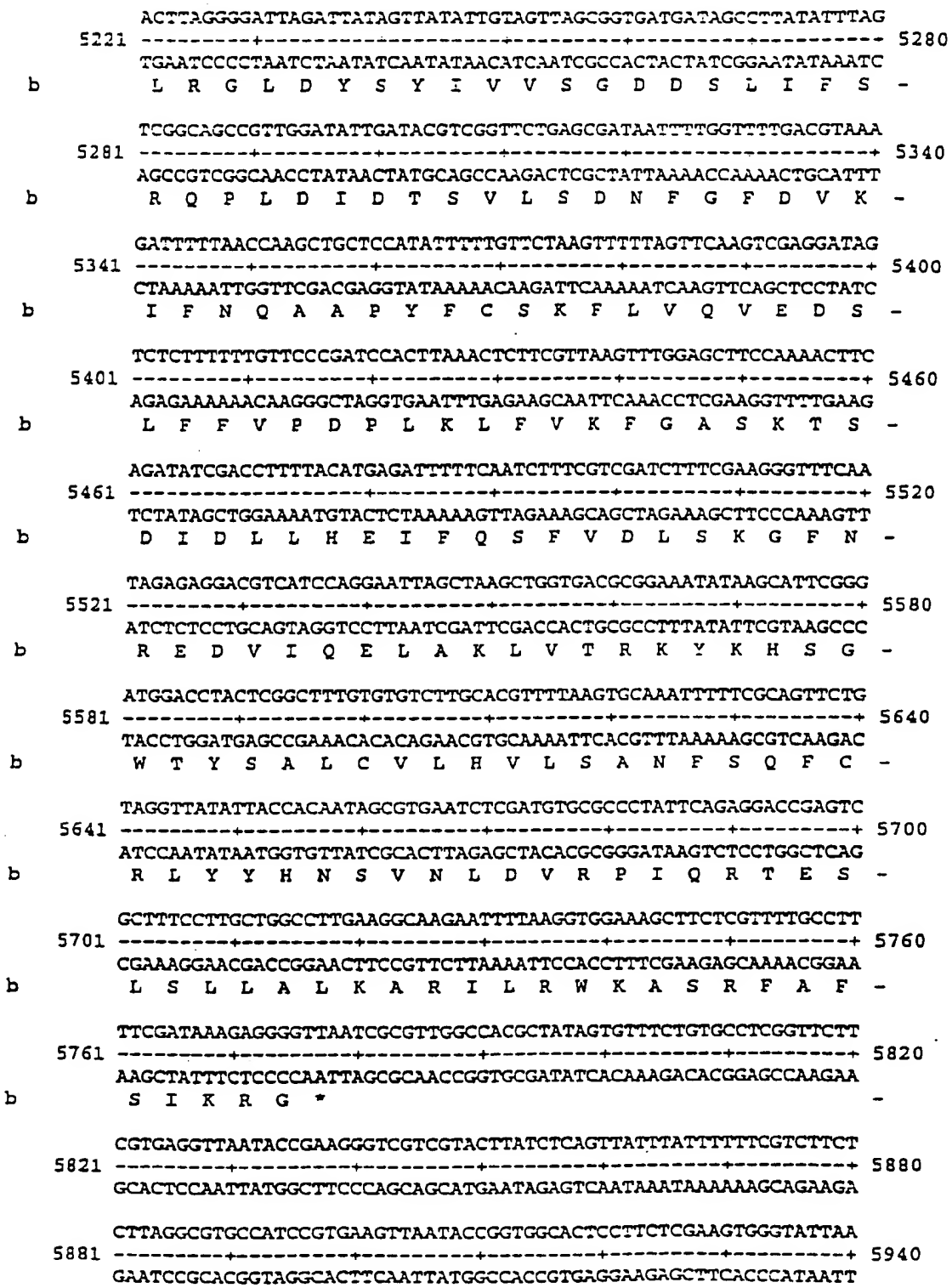
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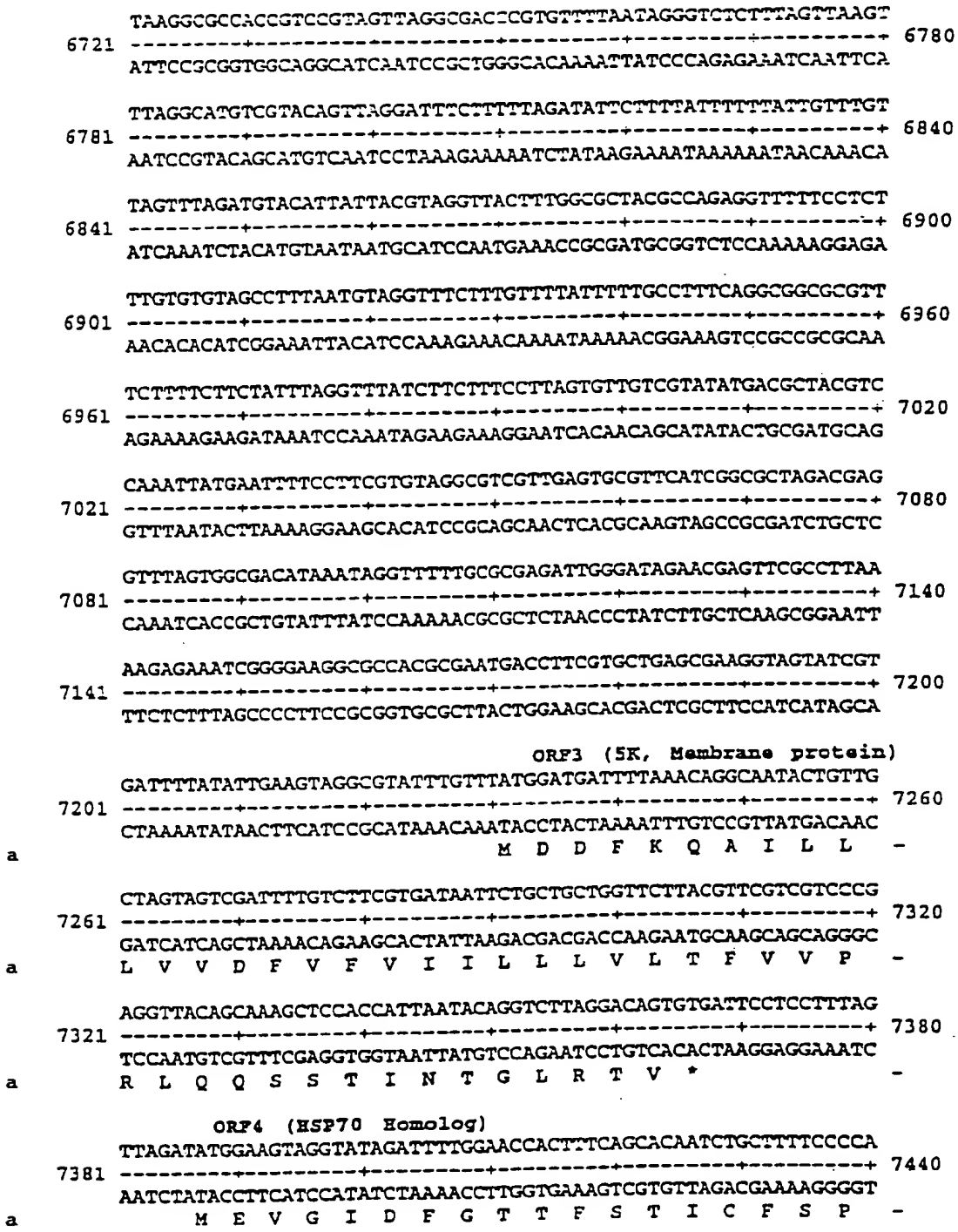
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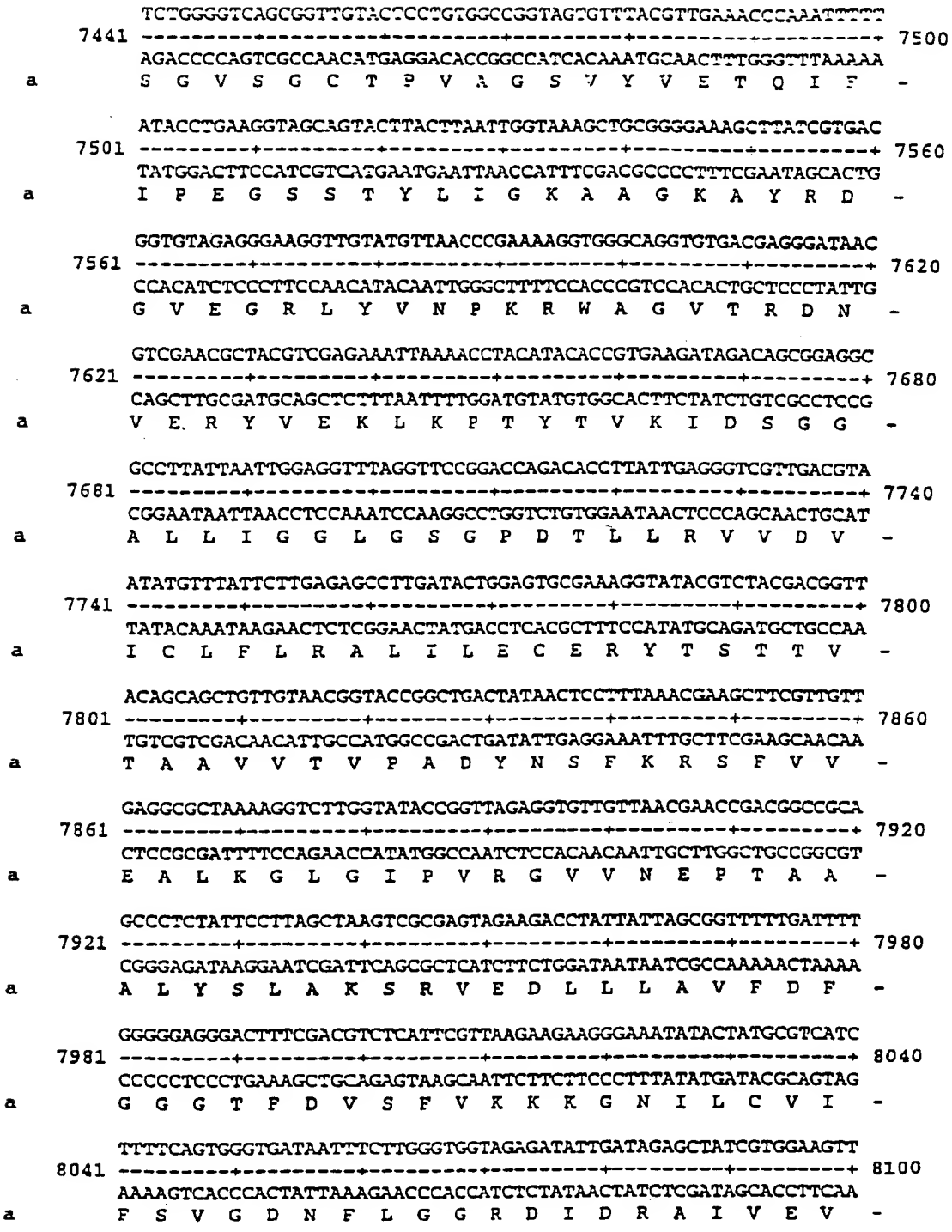
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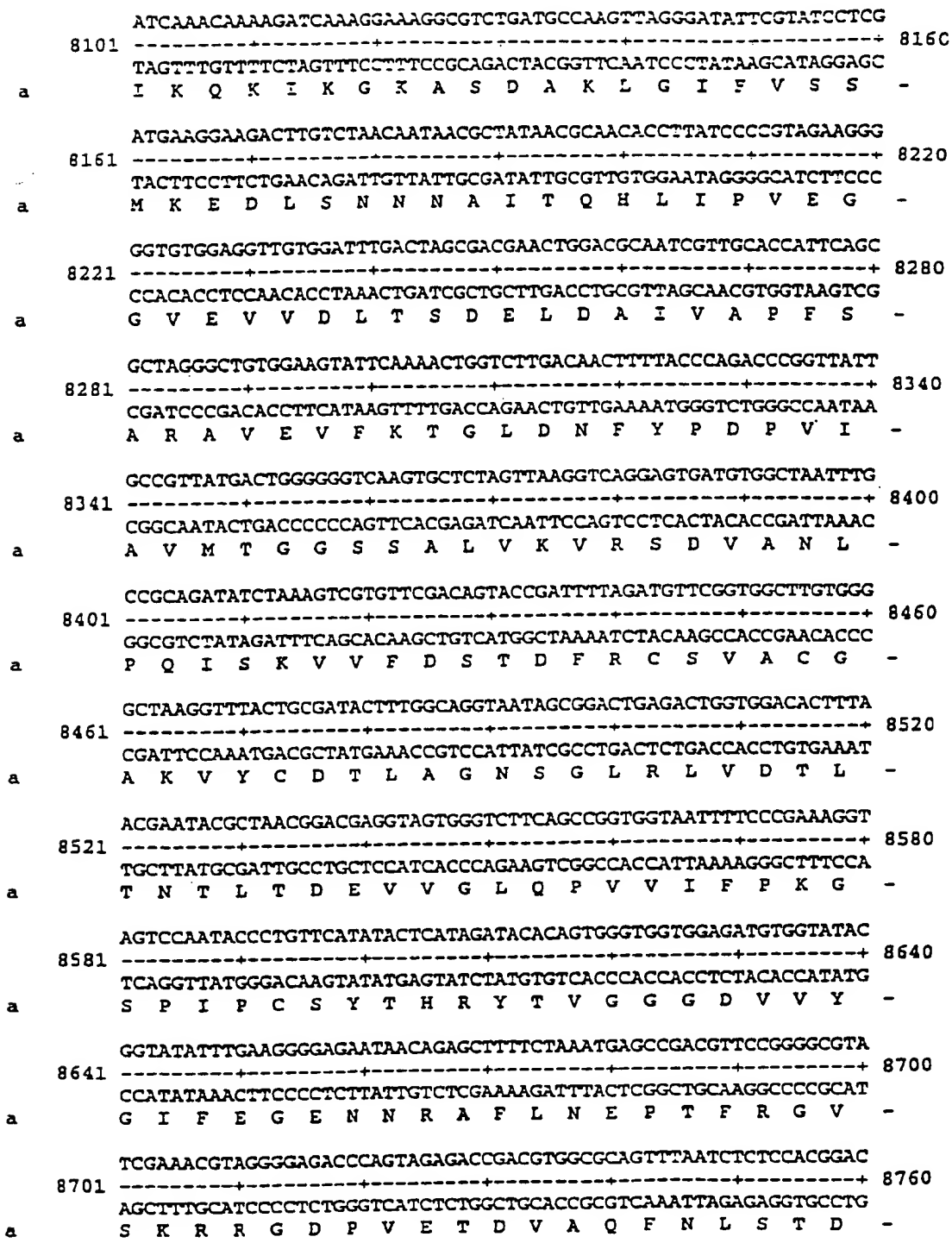
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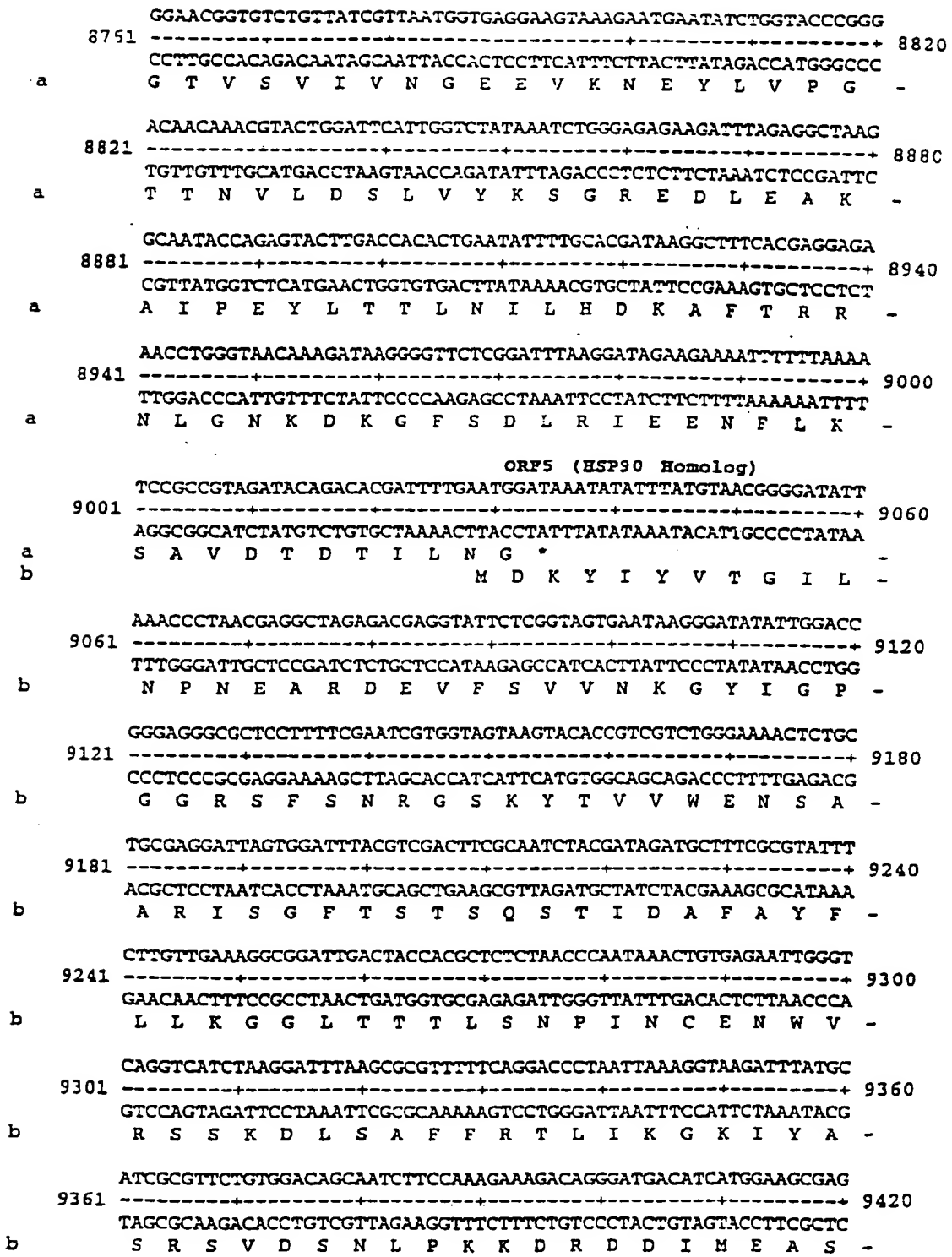
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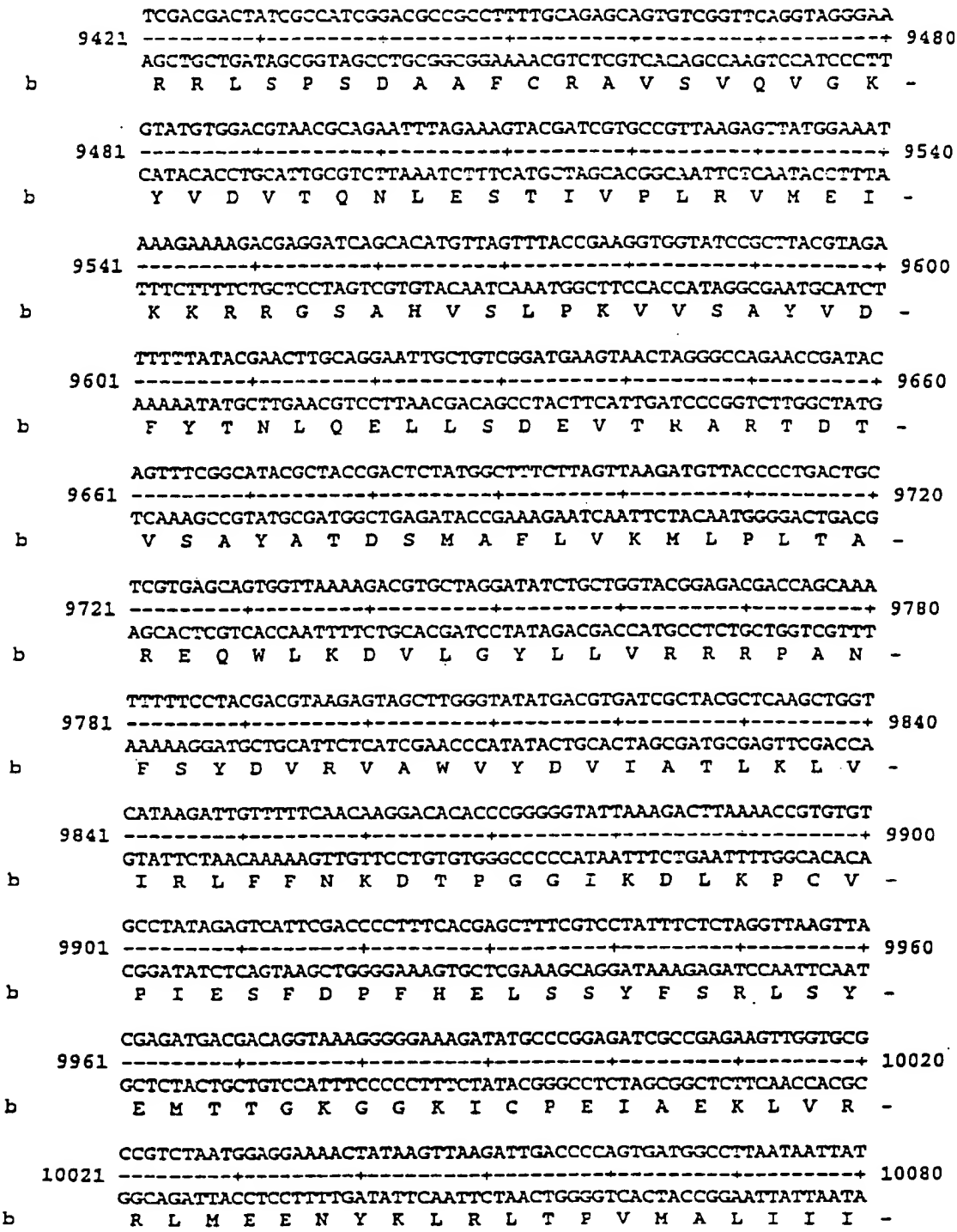
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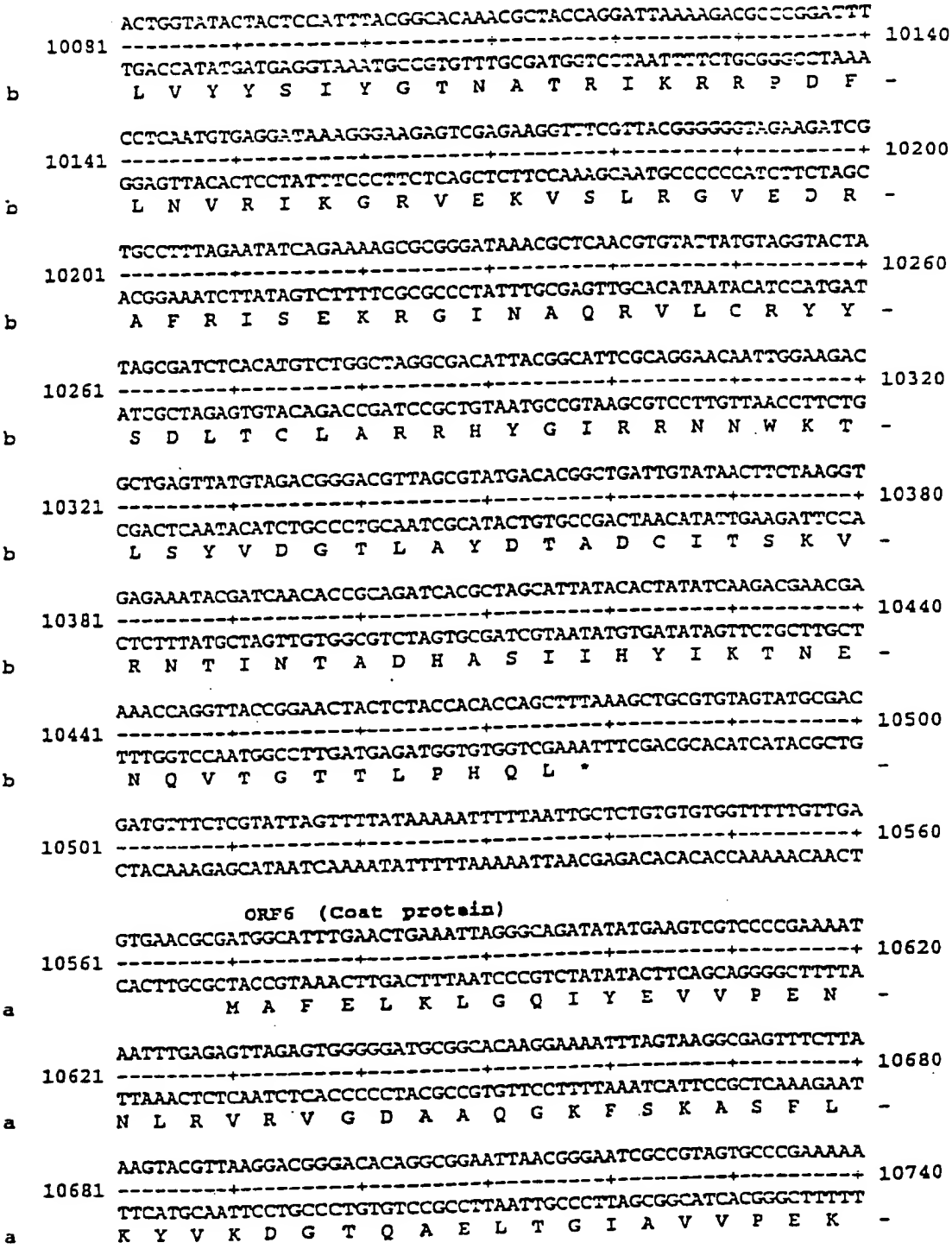
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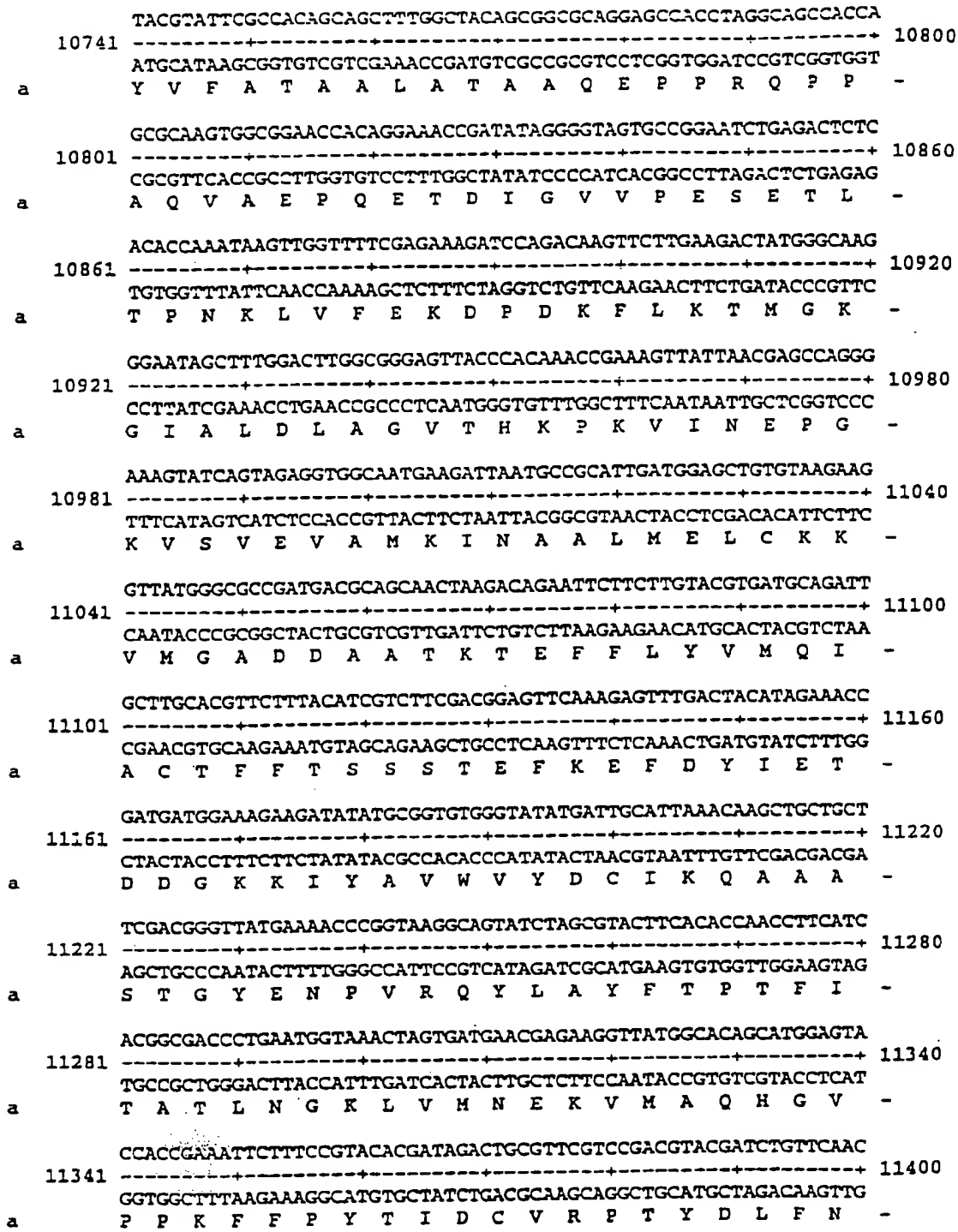
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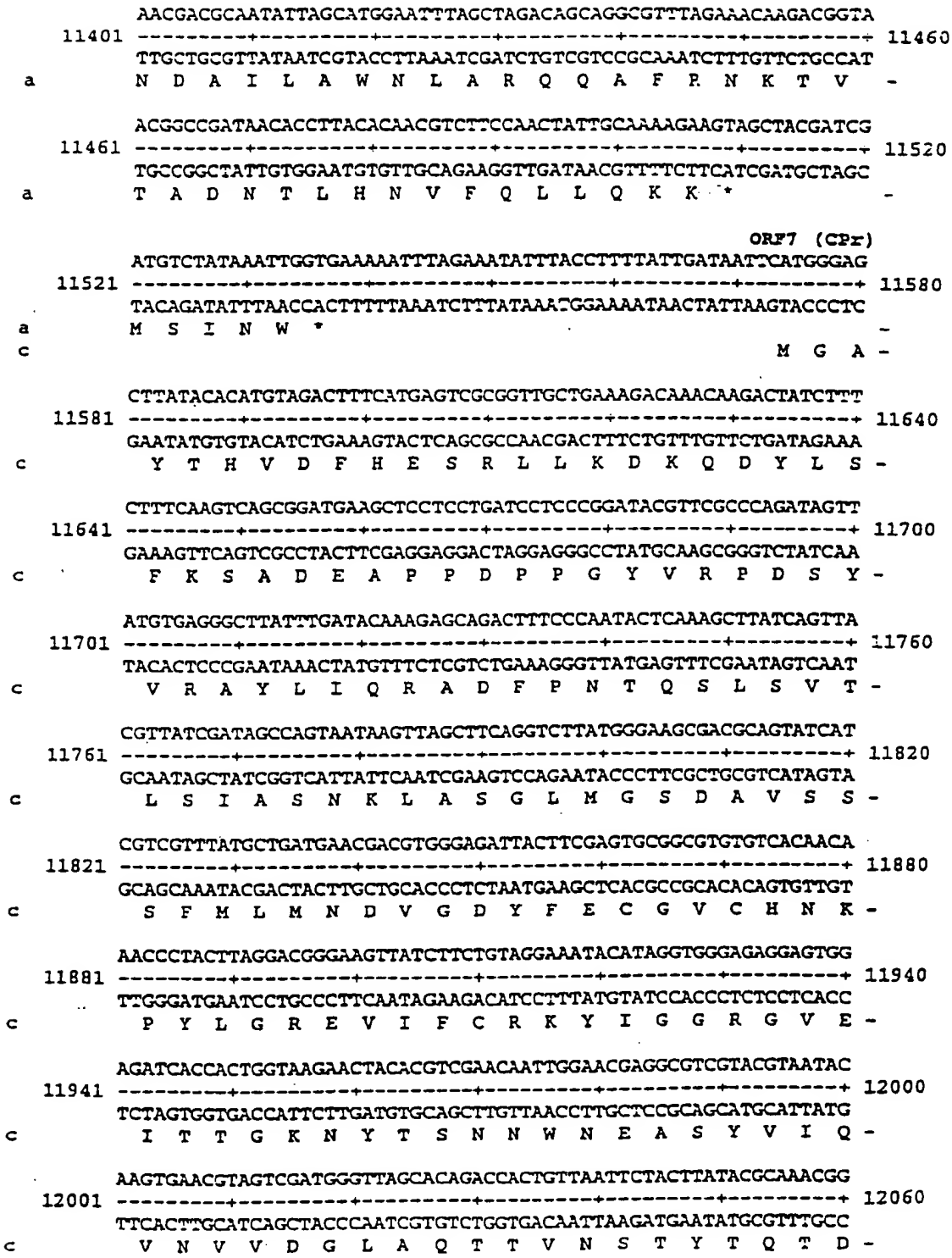
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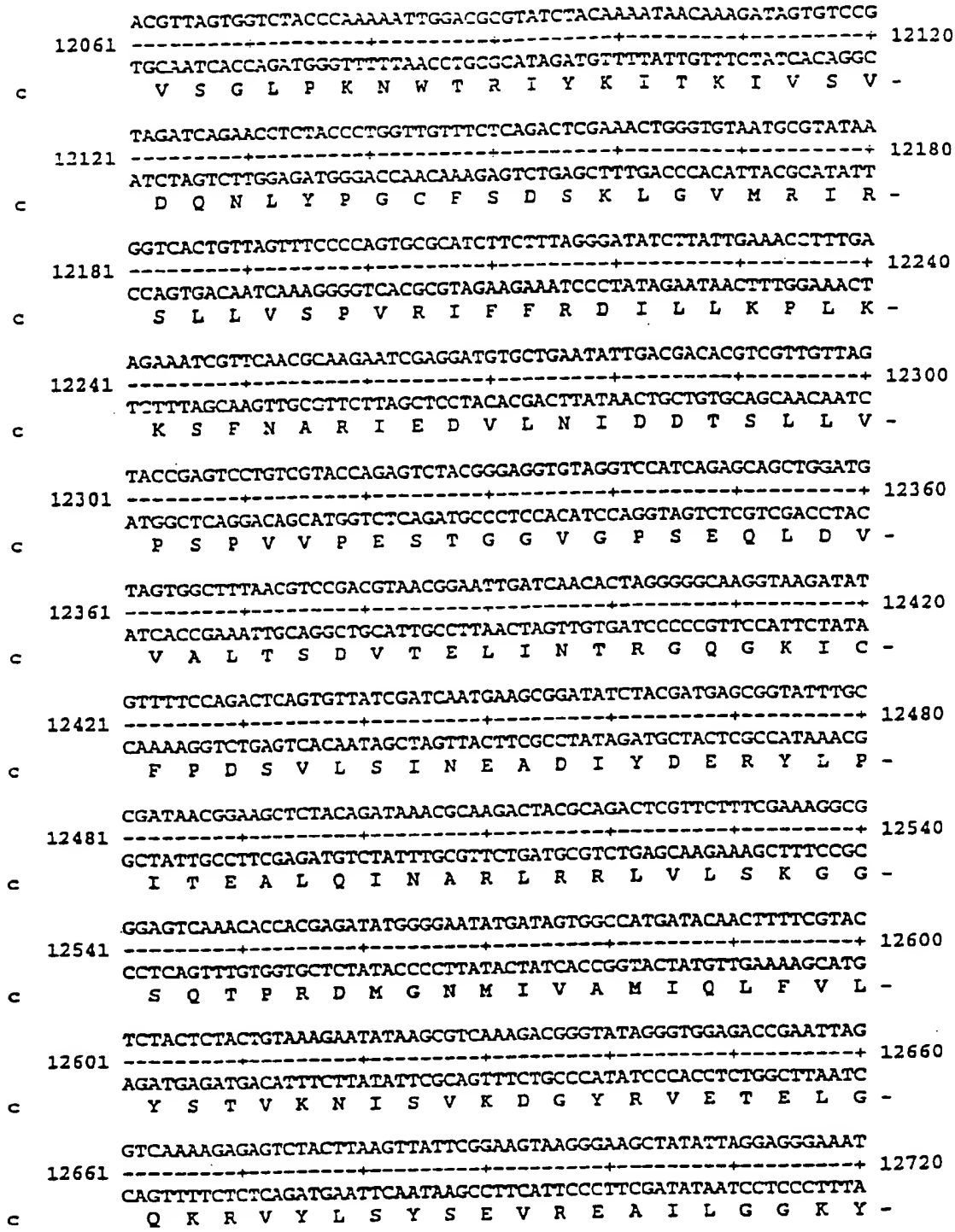
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12481
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12541
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12660
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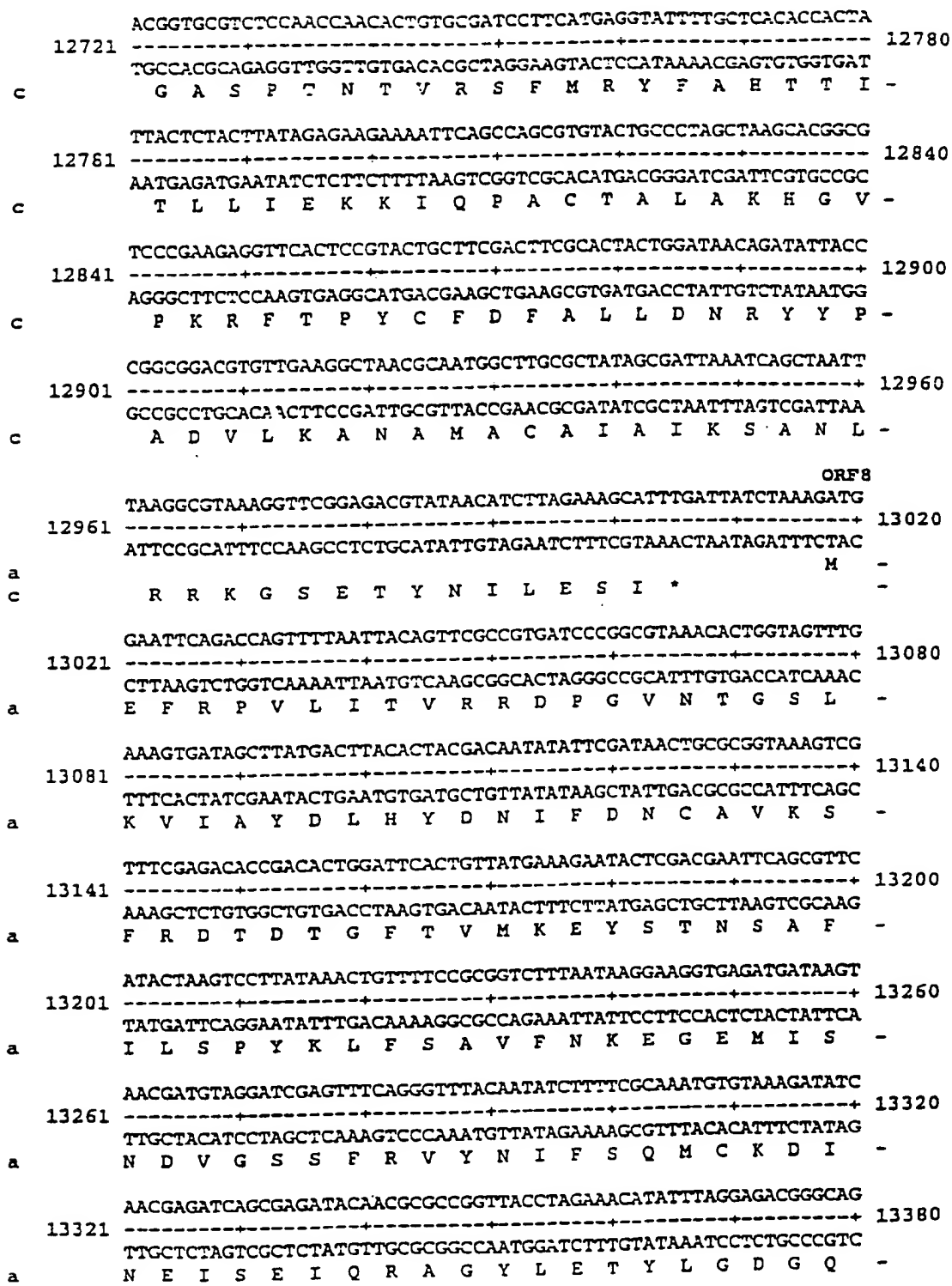
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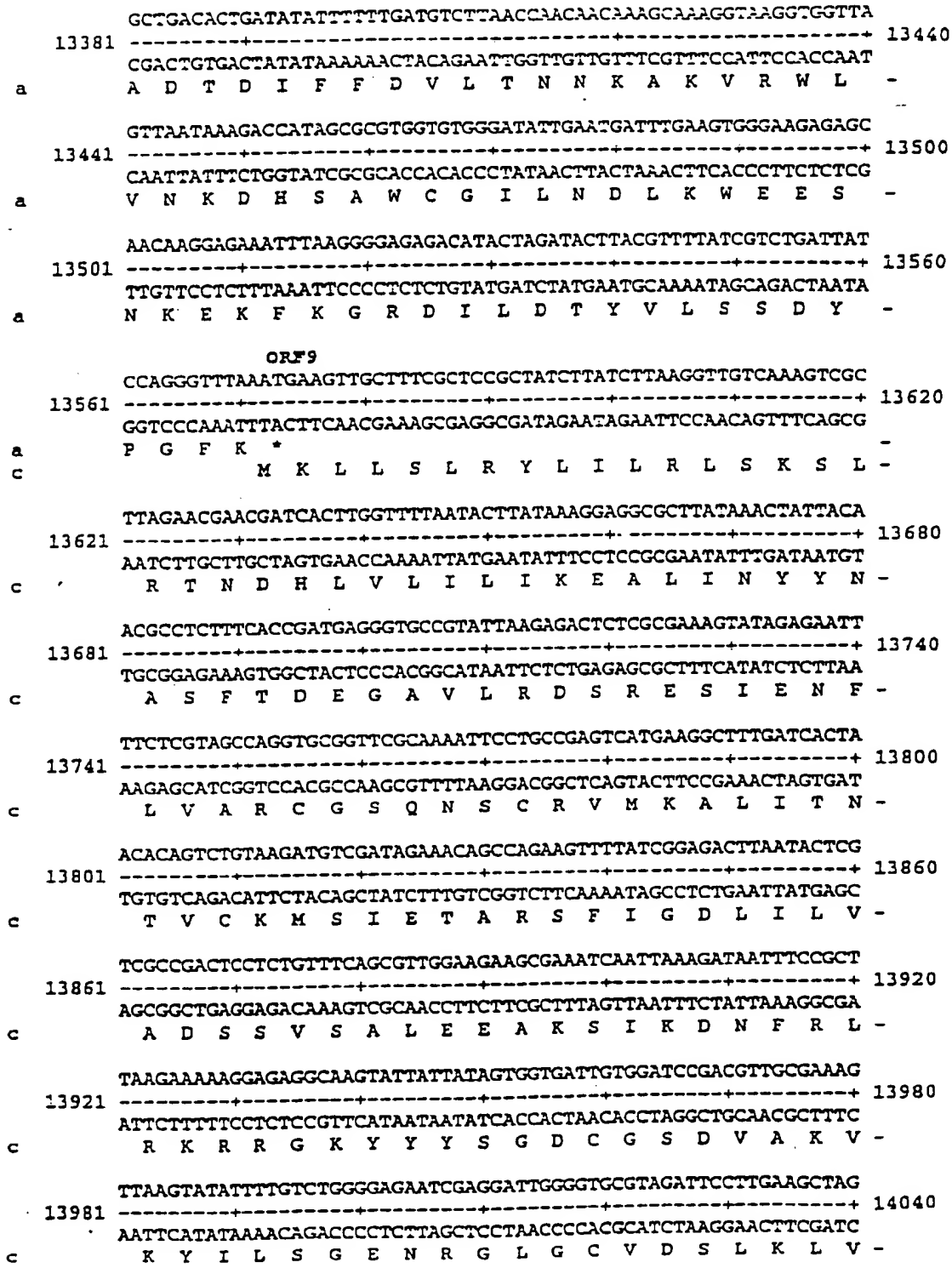
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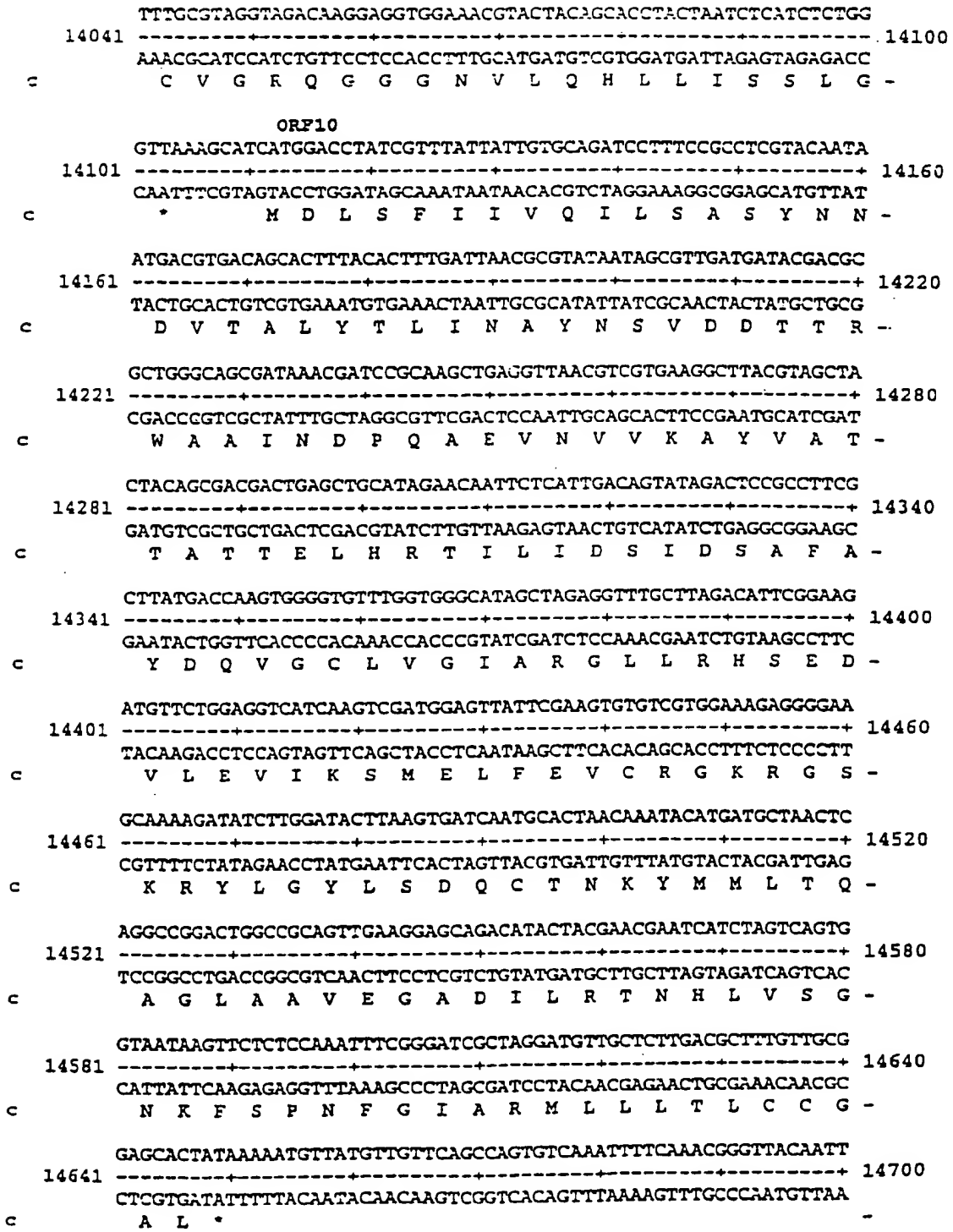
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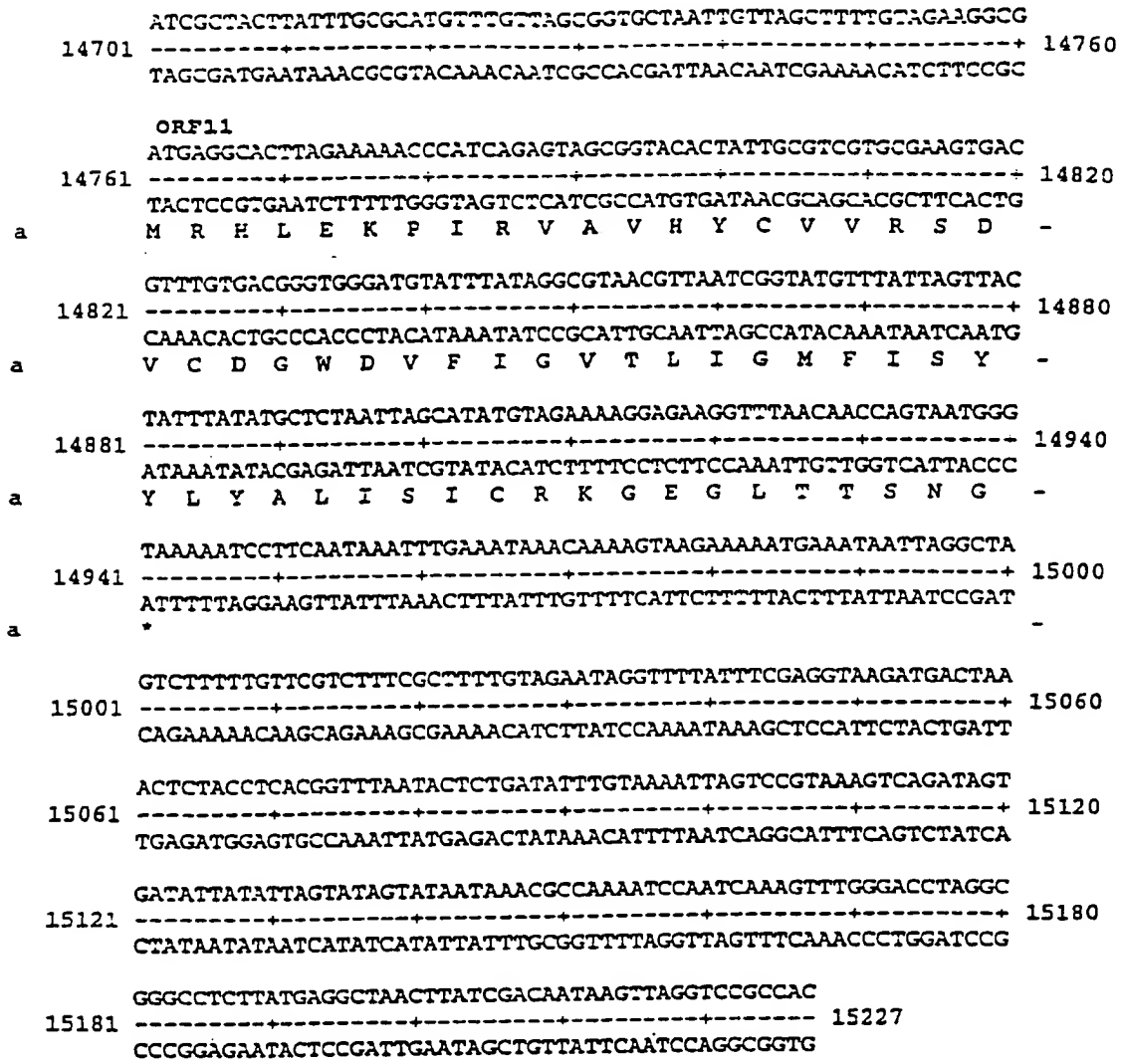
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14941 15000
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15061 15120
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15121 15180
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GGGCCTCTTATGAGGCTAACTTATCGACAATAAGTTAGGTCCGCCAC
15181 15227
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TABLE 5

Amino Acid Sequence Alignment for Helicases Encoded by GLRaV-3, BYV, CTV and LIYV.

```
                        _______I(A)_____                        _______Ia_____
   BYV_HEL  FTFTNLSANV LLYEAPPGGG KTTTLIKVFC ETFSK.VNSL ILTANKSSRE
   CTV_HEL  LTFTNEEHSL IVYEAPPGGG KTHSLVNSYA DYCVK.VSCL VVTANKNSQT
GLRaV3_HEL  VGESFKSFEY KCYNAPPGGG KTT....MLV DEFVKSPNST ATITANVGSS
  LIYV_HEL  MVRRPDVNGL KFYNKPPGAG KTTTIAKLMS KDLKNKVKCL ALSYTKVGRL

CONSENSUS  ---------- --Y-aPPGaG KTt------- d-f-k-v--l -----k----

                                                            ___II_
   BYV_HEL  EILAKVNRIV LD...EGDTP LQTRDRILTI DSYLMNNR.G LTCKVLYLDE
   CTV_HEL  EISQRISNEL MGRKLAAKYV TDAASRVFTV DSYLMNHL.R LTTQLLFIDE
GLRaV3_HEL  EDINM....A VKGR...DPN LEGLNSATTV NSRVVNFIVR GMYKRVLVDE
  LIYV_HEL  ELIDKLKKDG IEKP...EKY VKTYDSFLMN NDNILEIV.. ....NLYCDE

CONSENSUS  E--------- -------d-- -------ltv -s--mn---- -----ly-DE

                                  ___III___
   BYV_HEL  CFMVHAGAAV ACIEFTKCDS AILFGDSRQI RYGRCSELDT AVLSDLNRFV
   CTV_HEL  CFMVHAGAIG AVVEFTSCKA VVFFGDSKQI HYIHRNDLGV SFVADIDAFI
GLRaV3_HEL  VYMMHQGLLQ LGVFATGASE GLFFGDINQI PFINREKVFR MDCA..VFVP
  LIYV_HEL  VFMMHAGHFL TLLTKIAYQN GYCYGDVNQI PFINRDPYTP AYLS..REFF

CONSENSUS  -fM-HaG--- -------c-- --ffGD--QI --i-r----- --------f-

                     ____IV____
   BYV_HEL  DDESRVYGEV SYRCPWDVCA WLSTF..... ...YPKTVAT TNLVSAGQSS
   CTV_HEL  QPEHRIYGEV SYRCPWDICE WLSEF..... ...YPRHVAT ANVGSIGKSS
GLRaV3_HEL  KKESVVYTSK SYRCPLDVCY LLSSMTVRGT EKCYPEKVVS GKDK.PVVRS
  LIYV_HEL  RKQDLNYDTY TYRCPLDTCY LLSNLKDEMG NIIYAGGVKN VNEVYPTIRS

CONSENSUS  --e--vY--- sYRCP-DvC- -LS-f----- ---Yp--V-- -n-------S

                                                                  _
   BYV_HEL  MQVREIESVD DVEYSSEFVY LTMLQSEKKD LLKSFGK..R SRSSVEKPTV
   CTV_HEL  VSIEEINGCD DVPYDKAAKY IVYTQAEKND LQKHLGRLTV GRNKV.VPIV
GLRaV3_HEL  LSKRPIGTTD DVAEINADVY LCMTQLEKSD MKRSLKGKGK .ETP.....V
  LIYV_HEL  LNLFGINVVG EVPVEYNAKY LTFTQDEKLN LQRHIDSQGG CRNA.....V

CONSENSUS  l----I---d dV-------Y l--tQ-EK-d l---l----- -r-------V

                  _____V_____                        _____VI_____
   BYV_HEL  LTVHEAQGET YRKVNLVRTK FQEDDPFRSE NHITVALSRH VESLTYSVLS
   CTV_HEL  NTVHEVQGET YKRVRLVRFK YQEDTPFSSK NHIVVALTRH VDSLVYSVLT
GLRaV3_HEL  MTVHEAQGKT FSDVVLFRTK KADDSLFTKQ PHILVGLSRH TRSLVYAALS
  LIYV_HEL  STVNEAQGCT FSEVNLVRLV QFDNPVMSDI NQFVVAISRH TTTFKYFTPH

CONSENSUS  -TVhEaQG-T ---V-LvR-k ---d--f--- nhi-ValsRH --sl-Y--l-


   BYV_HEL  SKRDDAIAQA I
   CTV_HEL  SRRYDDTATN I
GLRaV3_HEL  SKLDDKVGTY I
  LIYV_HEL  SRLNDRVSNA I

CONSENSUS  S---D-v--- I
```

TABLE 6

	Helicase		RdRp		p5K		HSP70		HSP90		CP	
Virus	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
BYV	37.7	38.4 (58.1)	44.5	41.2 (61.0)	42.0	30.4 (47.8)	43.5	28.6 (48.0)	40.5	21.7 (51.0)	41.5	20.3 (43.7)
CTV	45.3	36.3 (55.2)	44.0	40.1 (62.2)	42.8	20.0 (48.9)	43.7	28.7 (49.3)	38.6	17.5 (43.5)	40.3	20.5 (41.9)
LIYV	44.9	32.4 (53.5)	46.2	35.9 (56.4)	45.8	17.9 (46.2)	43.9	28.2 (46.9)	39.3	16.7 (36.8)	36.3	17.8 (41.1)

Nucleotide (nt) and amino acid (aa) sequence similarity was calculated from perfect matches after aligning with the GCG program GAP; the percentages in parentheses are the percentages calculated by the GAP program, which employs a matching table based on evolutionary conservation of amino acids (Devereux et al., Nucleic Acids Res., 12:387-395 (1984), which is hereby incorporated by reference). The sources for the BYV, CTV, and LIYV sequences were, respectively, Agranovsky (1994), Karasev (1995), and Klaassen (1995), which are hereby incorporated by reference.

TABLE 7

Sample #	Accession #	ELISA *	RT-PCR	Indexing
1	476.01	1.424 (+)	+	+
2	447.01	0.970 (+)	+	+
3	123.01	1.101 (+)	+	+
4	387.01	>1.965 (+)	+	+
5	80.01	>2.020 (+)	+	+
6	244.01	>2.000 (+)	+	+
7	441.01	>2.000 (+)	+	+
8	510.01	0.857 (+)	+	+
9	536.01	0.561 (+)	+	+
10	572.01	>2.000 (+)	+	+
11	468.01	>2.000 (+)	+	+
12	382.01	>2.000 (+)	+	+
13	NY1	0.656 (+)	+	+
14	Healthy	0.002 (-)	-	-

Plus (+) and Minus (-) represent positive and negative reactions, respectively. For ELISA an OD_{405nm} that was at least twice higher than a healthy control, and more than 0.100 was regarded as positive.

TABLE 8

Amino Acid Sequence Alignment of RNA-dependent RNA Polymerase (RdRP) of GLRaV-3, BYV, CTV and LIYV.

```
                   ____I___                        ______________II_________
BYV_RdRp     ITTFKLMVKR DAKVKLDSSC LVKHPPAQNI MFHRKAVNAI FSPCFDEFKN
CTV_RdRp     ISNFKLMVKR DAKVKLDDSS LSKHPAAQNI MFHKKFINAI FSPCFDEFKN
GLRaV3_RdRp  LTSYTLMVKA DVKPKLDNTP LSKYVTGQNI VYHDRCVTAL FSCIFTACVE
LIYV_RdRp    FKTLNLMVKG ETKPKMDLST YDSYNAPANI VYYQQIVNLY FSPIFLECFA

CONSENSUS    ---f-LMVK- d-K-KlD-s- l-k----qNI --h---vna- FSp-F-e---

             ___        _____III____                       _____IV_____
BYV_RdRp     RVITCTNSNI VFFTEMTNST LASIAKEMLG .SEHVYNVGE IDFSKFDKSQ
CTV_RdRp     RVLSSLNDNI VFFTEMTNAG LAEIIRRIIG .DDDNLFVGE VDFSKFDKSQ
GLRaV3_RdRp  RLKYVVDERW LFYHGMDTAE LAXALRNNLG .DIRQYYTYE LDISKYDKSQ
LIYV_RdRp    RLTYCLSDKI VLYSGMNTDV LAELIESKLP LGLNAYHTLE IDFSKFDKSQ

CONSENSUS    R------d-i vf---M---- LA------lg -----y---E iDfSKfDKSQ

                                                        ___________
BYV_RdRp     DAFIKSFERT LYSAFGFDED LLD.VWMQGE YTSNATTLDG QLSFSVDNQR
CTV_RdRp     DLFIKEYERT LYSEFGFDTE LLD.VWMEGE YRARATTLDG QLSFSVDGQR
GLRaV3_RdRp  SALMKQVEEL ILLTLGVDRE VLS.TFFCGE YDSVVRTMTK ELVLSVGSQR
LIYV_RdRp    GTCFKLYEEM MYKMFGFSPE LYDRDFKYTE YFCRAKA.TC GVDLELGTQR

CONSENSUS    --f-K-yE-- ly--fGfd-e lld-----gE Y---a-tl-- -l--sv--QR

             _______V_____________            _____VI_____
BYV_RdRp     KSGASNTWIG NSIETLGILS MFYYTNRFKA LFVSGDDSLI FSESPIRNSA
CTV_RdRp     RSGGSNTWIG NSLVTLGILS LYYDVSKFDL LLVSGDDSLI YSSEKISNFS
GLRaV3_RdRp  RSGGANTWLG NSLVLCTLLS VVLRGLDYSY IVVSGDDSLI FSRQPLDIDT
LIYV_RdRp    RTGSPNTWLS NTLVTLGMML SSYDIDDIDL LLVSGDDSLI FSRKHLPNKT

CONSENSUS    rsG--NTW-G Nslvtlg-ls --y----f-- llVSGDDSLI fS-----n--

                                ___VII___      _____VIII_____
BYV_RdRp     DAMCTELGFE TKFLTPSVPY FCSKFFVMTG HDVFFVPDPY KLLVKLGAS.
CTV_RdRp     SEICLETGFE TKFMSPSVPY FCSKFVVQTG NKTCFVPDPY KLLVKLGAP.
GLRaV3_RdRp  SVLSDNFGFD VKIFNQAAPY FCSKFLVQVE DSLFFVPDPL KLFVKFGAS.
LIYV_RdRp    QEINKNFGME AKYIEKSSPY FCSKFIVELN GKLKVIPDPI RFFEKLSIPI

CONSENSUS    ------fGfe -Kf---s-PY FCSKF-V--- ----fvPDP- kl-vKlga--

BYV_RdRp     ..KDEVDDEF LFEVFTSFRD LTKDLVDERV IELLTHLVHS KYGYESGDTY
CTV_RdRp     ..QNKLTDVE LFELFTSFKD MTQDFGDQVV LEKLKLLVEA KYGFASGTTM
GLRaV3_RdRp  ..KTSDID.L LHEIFQSFVD LSKGFNREDV IQELAKLVTR KYK.HSGWTY
LIYV_RdRp    RQEDFVNGSV VKERFISFKD LMKEYDNDVA VIRIDEAVCY RYSIPVGCSY

CONSENSUS    -------d-- l-E-F-SF-D l-kdf--e-v i--l--lV-- kY---sG-ty

BYV_RdRp     AALCAIHCIR SNFSSFKKLY
CTV_RdRp     PALCAIHCVR SNFLSFERLF
GLRaV3_RdRp  SALCVLHVLS ANFSQFCRLY
LIYV_RdRp    AALCYIHCCM SNFVSFPRIY

CONSENSUS    -ALC-iHc-- sNF-sF-rly
```

TABLE 9

Nucleotide Sequence Comparison of GLRaV-3 and LIYV in Vicinity of Frameshift

GLRaV-3_HEL	S	P	Q	S	V	S	D	A	L	L												
								GLRaV-3_RdRp			T	R	S	P	R	L	V	A	F	E	V	Y
GLRaV-3 (nt)	TCg	Cct	CAA	TCa	GTa	tcc	GAc	ncT tTg cTtc			Aca	cgT	tcg	ccc	ccg	ctg	gtT	GCt	TTc	GAg	GTa	TAT
LIYV (nt)	TCa	Cta	CAA	TCt	GTt	agt	GAt	tcT gTt tTga			Aag	acT	atc	att	tta	gac	agT	GCc	TTt	GAc	GTg	TAT
LIYV_HEL	S	L	Q	S	V	S	D	F	V	L												
								LIYV_RdRp			K	T	I	I	L	D	S	A	F	D	V	Y

TABLE 10

Amino Acid Sequence Alignment of Small Hydrophobic Transmembrane Proteins of GLRaV-3 (p5k) and BYV (p6K), CTV (p6K) and LIYV (p5K).

	1	Transmembrane	54
BYV_p7K	MDCVLRSYLL	LAFGFLICLF LFCLVVFIWF VYKQILFRTT AQSNEARHNH	STVV*
LIYV_P5K	.....	..MSILLFFL MSILVWFIFT ILKLLFVNID SEVNIPNRSR	F*...
GLRaV3_p5K	.....MDD	FKQAILLLVV DFVFVIILLL VLTFVVFRLQ QSSTINYGLR	TV*..
CTV_p6K	MDCVIQGFLT	FLVGIAVFCA FAGLIIIVIT IYRCTIKPVR SASPYGTHAT	V*...
CONSENSUS	----------	f---il-f-- ---lvi-i-- ---------- --s-------	----

TABLE 11

Amino Acid Sequence Alignment of ASP70-related protein of GLRaV-3 with BYV (p65K), CTV (p65K) and LIYV (p62K).

```
                 _____A_____
BYV_p65   ..MVVFGLDF GTTFSSVCAY VGEELYLFKQ RDSAYIPTYV FLESDTQEVA
CTV_p65   ..MVLLGLDF GTTFSTVAMA TPSELVILKQ SNSSYIPTCL LLEAEPNSVS
GLRaV3_p59 ...MEVGIDF GTTFSTICFS PSGVSGCTPV AGSVYVETQI FIPEGSSTYL
LIYV_p62  MRDCKVGLDF GTTFSTVSTL VNNSMYVLRL GDSAYIPTCI AITPGGEAI.

CONSENSUS ------GlDF GTTFStv--- ----l--l-- --S-YipTci f-------v-

BYV_p65   FGYDAEVLSN DLSVRGGFYR DLKRWIGCDE ENYRDYLEKL KPHYKTELLK
CTV_p65   YGYDAEYLAA S.GESGSFYK DLKRWVGCTA KNYQTYLHKL SPSYKVIVKE
GLRaV3_p59 IG.KAAGKAY RDGVEGRLYV NPKRWVGVTR DNVERYVEKL KPTYTVKM..
LIYV_p62  IGGAAEVLSG DDTPHCFFY. DLKRWVGVDD NTFKFAMNKI RPKYVAELVE

CONSENSUS -G--Ae-l-- -----g-fY- dlKRWvG--- -ny--yl-Kl -P-Y---l--

BYV_p65   VAQSSKSTVK LDCYSGTVPQ NATLPGLIAT FVKALISTAS EAFKCQCTGV
CTV_p65   FGTKSVPVPY LSPLNNDLGL SVALPSLIAS YAKSILSDAE RVFNVSCTGV
GLRaV3_p59 ...DSGGALL IGGLGSGPDT LLRVVDVICL FLRALILECE RYTSTTVTAA
LIYV_p62  ......GEVY LTGINKGFSI KLSVKQLIKA YIETIVRLLA SSYSLRVIDL

CONSENSUS ----s----- l--------- ------lI-- -----i---- --f----T--

                 _____________________B_____________________
BYV_p65   ICSVPANYNC LQRSFTESCV NLSGYPCVYM VNEPSAAALS ACSRIKGATS
CTV_p65   ICSVPAGYNT LQRAFTQQSI SMSGYSCVYI INEPSAAAYS TLPKLNSADK
GLRaV3_p59 VVTVPADYNS FKRSFVVEAL KGLGIPVRGV VNEPTAAALY SLAKSRVEDL
LIYV_p62  NQSVPADYKN AQRLAARSVL KALSFPCRRI INEPSAAAVY CVSRYPNYNY

CONSENSUS i-sVPA-Yn- lqR-f----- ---gypc--i -NEPsAAA-- ----------

          _____C_____          _____________D_____________
BYV_p65   PVLVYDFGGG TFDVSVISAL NNTFVVRASG GDMNLGGRDI DKAFVEHLYN
CTV_p65   YLAVYDFGGG TFDVSIVSVR LPTFAVRSSS GDMNLGGRDI DKKLSDKIYE
GLRaV3_p59 LLAVFDFGGG TFDVSFVKGK GNILCVIFSV GDNFLGGRDI DRAIVEVIKQ
LIYV_p62  FL.VYDFGGG TFDVSLIGKY KSYVTVIDTE GDSFLGGRDI DKSIEDYLVG

CONSENSUS -l-VyDFGGG TFDVS----- ---f-V--s- GD--LGGRDI Dk--------

BYV_p65   KAQ...LPVN YKIDISFLKE SLSKKVSFLN FPVVSEQGVR VDVLVNVSEL
CTV_p65   MAD...FVPQ KELNVSSLKE ALSLQTDPVK YT.VNHYGMS ETVSIDQTVL
GLRaV3_p59 KIKGKASDAK LGIFVSSMKE DLSNNNAITQ HLIPVEGGVE V.VDLTSDEL
LIYV_p62  KYNIKKVIP. .ATYLALIKE E.CNNTNKSI FTILFDDGSV QVVEFSKSEL

CONSENSUS k--------- ----vs-lKE -ls------- f-i--e-G-- --V-----eL

                                                  _____E_____
BYV_p65   AEVAAPFVER TIKIVKEVY. .EKYCSSMRL EPNVKAKLLM VGGSSYLPGL
CTV_p65   REIASVFINR TIDILTQV.. ..KVKSSMPE SQSL..KLVV VGGSSYLPGL
GLRaV3_p59 DAIVAPFSAR AVEVFKTGP. .DNFYPDPVI A.......VM TGGSSALVKV
LIYV_p62  EKCVRPFVER SIKLINDVVV RNKLTSGV.. .......IYM VGGSSLLQPV

CONSENSUS --i--pFv-R -i-i---v-- --k--s---- ---------m vGGSS-L---
```

```
                                             __________F__________   __________
BYV_p65     LSRLSSIPFV DEC.L.VLPD ARAAVAGGCA LYSACLANDS PMLLVDCAAH
CTV_p65     LDALATVPFV SGI.V.PVED ARTAVARGCA LYSECLDGRS KALLIDCITH
GLRaV3_p59  RSDVANLPQI SKV.VFDSTD FRCSVACGAK VYCDTLAGNS GLRLVDTLTN
LIYV_p62    QDMVRSYAST KGLTLVADQD MRSAVSYGCS VLHK.LEDNK EIVYIDCNSH

CONSENSUS   -------p-v ---------D -R-aVa-Gc- -y---L---s ---l-Dc--h

            ____G__________
BYV_p65     NLSISSKYCE SIVCVPAGSP IPFTGVRTVN MTGSNASAVY SAALFEGDFV
CTV_p65     HLSVTTFSAD SVVVAAAGSP IPFEGERKLT LRKCVSTSNY QARMFEGDYE
GLRaV3_p59  TLTDEVVGLQ PVVIFPKGSP IPCSYTHRYT V....GGGDV VYGIFEGE..
LIYV_p62    PLSDISFNCD PEPIIIKPMS IPYTHTVKMR HDRPLKT... IVNIYEGSNL

CONSENSUS   -Ls------d -vvi---gsp IPf------- ---------- ----fEGd--

                                     ________H_______
BYV_p65     KCRLNKRIFF GDVVLGNVGV TGSATRTVPL TLEINVSSVG TISFSLVGPT
CTV_p65     KVFRNERIYA ASVSLFTLGV NWSVPNDVEM TLVTKVDSMG KVEFYLKGPS
GLRaV3_p59  ....NNRAFL NEPTFRGVSK RRGDPVETDV A.QFNLSTDG TVSVIVNGEE
LIYV_p62    FMPENDWLIS SNINTTDFAK .....VGEEY SKVYEYDIDG IITLKIRNEV

CONSENSUS   ----N-r-f- --v-l----- --------e- ---------G ---f---g--

BYV_p65     GVKKLIGGNA AYDFSSYQLG ERVVADLHKH NSDKVKLIHA LTYQPFQRKK
CTV_p65     GELVNVQGTS HYDYAGMPHP TRKLVRLSDY NVNSAALVLA LTLTREKREK
GLRaV3_p59  VKNEYLVPGT TNVLDSL... ...VYKSGRE DLEAKAIPEY LTTLNILHDK
LIYV_p62    TGKMFTLPNS FTKSDNIKPI TFKLTQLSNT D.DLATLTSL LGYHDKNFER

CONSENSUS   ---------- ---------- ------l--- --d---l--- Lt------ek

BYV_p65     LTDGDKALFL KRLTADYRRE ARKFSSY... ......DDAV LNSSELLLGR
CTV_p65     FLLRT...LF DTLLADLRKT A.SLSEYSKK YPITRNDIDV VSSR...MGI
GLRaV3_p59  AFTRRNLGNK DKGFSDLRIE ENFLKS.... ...AVDTDTI LNG*......
LIYV_p62    FYG......L FNVPTILIKE IDKLGGFKTL YRRLKSMNAN F.........

CONSENSUS   f--------l -----dlr-e ---l--y--- ---------v l---------

BYV_p65     IIPKILRGSR VEKLDV*
CTV_p65     VVSKVLRGSD LERIPL.
GLRaV3_p59  .......... .......
LIYV_p62    .......... .......

CONSENSUS   ---------- -------
```

TABLE 12

Amino Acid Sequence Alignment of HSP90-like Protein (p55) of GLRaV-3 with p61K of CTV, p59 of LIYV and P64 of BYV.

```
   BYV_p64  115_VGCKFNIQSVTEFVKKINGNVAEPSLVEHCWSLSNSCGELINPKDTKRFV
   CTV_p61  108_VGCRFTLNDVESYLMSRGEDFADLAAVEHSWCLSNSCSRLLSSTEIDANK
  LIYV_P59  131_EGCSFTEQQVVEKYPQVDSLVAKIL.....YRVCNSLGKLLDLKDFENKN
GLRaV3_p55  114_VDSNLPKKDRDDIME..ASRRLSPSDAAFCRAVSVQVGKYVDVTQNLEST

 CONSENSUS      vgc-f----v-e---------a--------w--sns-g-l----d-----

   BYV_p64      SLIFKGKDLAESTDEAIVS..SSYLDYLSHCLNLYETCNLSSNSGKKSLY
   CTV_p61      TLVF.TKNFDSNISG..VT..TKLETYLSYCISLYKKHCM.KDDDYFNLI
  LIYV_P59      ISGFEINTAQDSPTVADDN..ES.NDFFRECVNDQRYYSSLSGSKLGKAK
GLRaV3_p55      IVPLRVMEIKKRRGSAHVSLPKVVSAYVDFYTNLQELLSDEVTRARTDTV

 CONSENSUS      ---f-----------a-v--------yl--c-nl----------------

   BYV_p64      DEFLKHVIDYL...ENSDLEYRSPSDNPLVAGILYDMCFEYNTLKSTYLK
   CTV_p61      LPMFNCLMKVL...ASLGLFYEKHADNPLLTGMLIEFCLENKVYYSTFKV
  LIYV_P59      LEANAYIFKILLKSASGEFDIDRLSRNPLAISKFMNLYTNHVTDSETFKS
GLRaV3_p55      SAYATDSMAFLVKMLPLT......AREQWLKDVLGYLLVRRRPANFSYDV

 CONSENSUS      ----------L-------l-------npl----l--lc--------t---

   BYV_p64      NIESFDCFLSLYLPLLSEVFSMNWERPAPDVRLLFELDAAELLLKVPTIN
   CTV_p61      NLDNVRLFKSKVLPVVLTVWDISEPDDPVDERVLIPFDPTDFVLDLPKLN
  LIYV_P59      KFEALKSIKTPFASFIKKAFGIR..........LNFEDSKIFYALPKER
GLRaV3_p55      RVAWVYDVIATLKLVIRLFFNKDTPGGIKDLKPCVPIESFDPFHELS...

 CONSENSUS      --e-------------i---f---------d------f---d-f--lp---

   BYV_p64      MHDST...FLYKNKLRYLESYFEDDSNELIKVKVDSLL
   CTV_p61      IHDTM...VVVGNQIRQLEYVVESDALDDLSQHVDLRL
  LIYV_P59      QSDVLSDDMMVESIVRDAASFTVVSDNNYLPERVDRFV
GLRaV3_p55      ............SYFSRLSYEMTTGKGGKICPEIAEKL

 CONSENSUS      --d-------------r-l---------------vd--l
```

TABLE 13

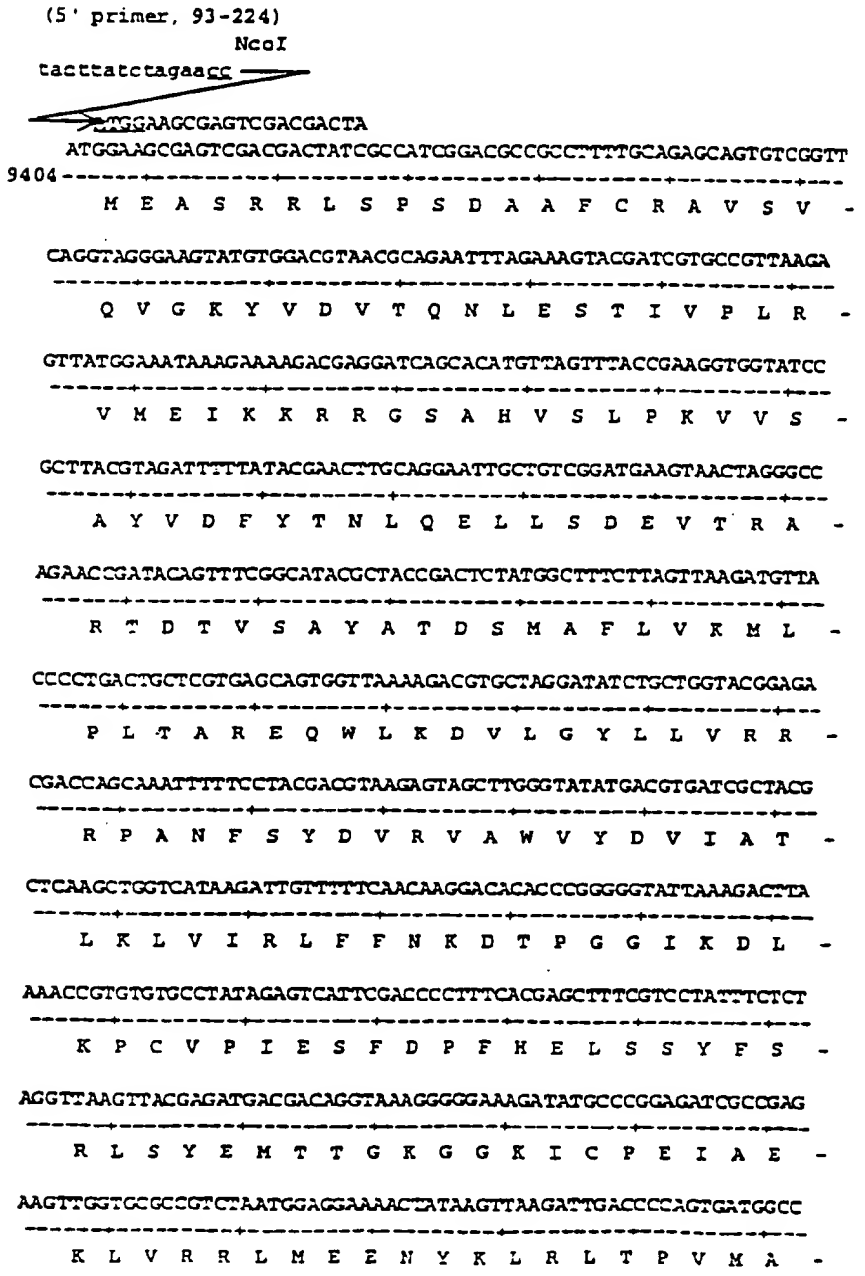
Amino Acid Sequence Alignment of the HSP90-related Protein of GLRaV-3 with BYV p64K, CTV p61K and LIYV p59K.

```
BYV_p61      MTTRFSTPAN YYWGELFRRF FGGQEW.... ....KNLMSE AASVSRPRYS
CTV_p61      ......MSSH HVWGSLFRKF YGEAIW.... ....KEYLSE STRNFDERNV
LIYV_P59     ..MLNDRIAV TCFQTLLKKS NVKHEMEQTN NYIVNNLADI NRNTFPALAG
GLRaV3_p55   .......MDK YIYVTGI..L NPNEARDEVF SVVNKGYIGP GGRSFSNRGS

CONSENSUS    ---------- --w--lf--f -----w---- ----k----- ----f--r--

BYV_p61      S.DFRFSDGV ILSRKTFGES TGES..FVRE FSLLLTFPKT YEVCKLCGVA
CTV_p61      SLDHTLSSGV VVRRQSLLNA PQGT..FENE LALLYNSVVI NDFVELTGMP
LIYV_P59     SVRIDFNSDY YISGGQIVVS PKDSNAYVKL LIVYLKYCYI N.YSAKTKYP
GLRaV3_p55   KYTVVWEN.. ..SAARISGF TSTSQSTIDA FAYFL..... ..LKGGLTTT

CONSENSUS    s----f---- --s------- ---s--fv-- ---ll----- ----------

BYV_p61      MELALNGMN. .RLSDYNVSE FN......IV DVKTVGCKFN IQSVTEFVKK
CTV_p61      LKSLMTGIED RKVPD....E LI......SV DPHEVGCRFT LNDVESYLMS
LIYV_P59     PQSLLAVLDY DSFKAKWVKY LDKSLTDYLD DNKTEGCSFT EQQVVEKYPQ
GLRaV3_p55   LSNPINCENW VRSSKDLSAF FRTLIKGKIY ASRSVDSNLP KKDRDDIME.

CONSENSUS    l---l----- ---------- ---------- d---vgc-f- ---v-e----

BYV_p61      INGNVAEPSL VEHCHSLSNS CGELINPKDT KRFVSLIFKG KDLAESTDEA
CTV_p61      RGEDFADLAA VEHSWCLSNS CSRLLSSTEI DANKTLVF.T KNFDSNISG.
LIYV_P59     VDSLVAKIL. ....YRVCNS LGKLLDLKDF ENKNISGFEI NTAQDSPTVA
GLRaV3_p55   .ASRRLSPSD AAFCRAVSVQ VGKYVDVTQN LESTIVPLKV MEIKKRRGSA

.CONSENSUS   -----a---- ----w--sns -g-l----d- -------f-- ---------a

BYV_p61      IVS..SSYLD YLSHCLNLYE TCNLSSNSGK KSLYDEFLKH VIDYL...EN
CTV_p61      .VT..TKLET YLSYCISLYK KHCM.KDDDY FNLILPMFNC LMKVL...AS
LIYV_P59     DDN..ES.ND FFRECVNDQR YYSSLSGSKL GKAKLEANAY IFKILLKSAS
GLRaV3_p55   HVSLPKVVSA YVDFYTNLQE LLSDEVTRAR TDTVSAYATD SMAFLVKMLP

CONSENSUS    -v-------- yl--c-nl-- ---------- ---------- ----L-----

BYV_p61      SDLEYRSPSD NPLVAGILYD MCFEYNTLKS TYLKNIESFD CFLSLYLPLL
CTV_p61      LGLFYEKHAD NPLLTGMLIE FCLENKVYYS TFKVNLDNVR LFKSKVLPVV
LIYV_P59     GEFDIDRLSR NPLAISKFMN LYTNHVTDSE TFKSKFEALK SIKTPFASFI
GLRaV3_p55   LT......AR EQWLKDVLGY LLVRRRPANF SYDVRVAWVY DVIATLKLVI

CONSENSUS    --l------- npl----l-- lc-------- t-----e--- ---------i

                                 I
BYV_p61      SEVFSMNWER PAPDVRLLFE LDAAELLLKV PTINMHDST. ..FLYKNKLR
CTV_p61      LTVWDISEPD DPVDERVLIP FDPTDFVLDL PKLNIHDTM. ..VVVGNQIR
LIYV_P59     KKAFGIR... ........LN FEDSKIFYAL PKERQSDVLS DDMMVESIVR
GLRaV3_p55   RLFFNKDTPG GIKDLKPCVP IESFDPFHEL S......... ......SYFS

CONSENSUS    ---f------ ---d------ f---d-f--l p-----d--- ---------r
```

```
BYV_p61       YLESYFEDDS NELIKVKVDS LLTRDNPEL. .KLAQRWV.. ...GFHCYYG
CTV_p61       QLEYVVESDA LDDLSQHVDL RLAADNPDL. .RVGLRWA.. ...GMFVYYG
LIYV_P59      DAASFTVVSD NNYLPERVDR FVTQLLLELF PKTKASFPNK IMFGFLHYFA
GLRaV3_p55    RLSYEMTTGK GGKICPEIAE KLVRRLMEEN YKLRLT.PVM ALIIILVYYS

CONSENSUS     -l-------- -------vd- -l-----el- -k----w--- ---g-l-Yy-

                                                  II
BYV_p61       VFRTAQTRKV KRDAEYKLPP AL......GE FVINMSGVEE FF.EELQKKM
CTV_p61       VYRCVVDRAV ERPTLFRLPQ KLLSQDDGES CSLHMGSVEA LF.NLVQKVN
LIYV_P59      LSTTNSKR.. .....FNDTQ ESTIEIEGET LKISLKFITS YLRNAIQSQH
GLRaV3_p55    IYGTNATRIK RRPDFLNVRI KGRVE..... .KVSLRGVED ..RAFRISEK

CONSENSUS     vy-t---R-- -r---f---- ---------- --i----ve- -f----q---

BYV_p61       PSI...SVRR RFCGSLSHEA FSVFKRFGVG FPPITRLNVP VKYSYLNVDY
CTV_p61       KDI...NVRR QFMGRHSEVA LRLYRNLGLR FPPISSVRLP AHHGYLYVDF
LIYV_P59      PDYADSNIVR LWCNKRSNLA LGYFKSRNIQ LYLYS..KYP RLLNYMRFDY
GLRaV3_p56    RGINAQRVLC RYYSDLTCLA RRHYGIRRNN WKTLSYVD.. GTLAYDTADC

CONSENSUS     --i----v-r -fc---s--A l--------- f---s----p ----Yl--Dy

BYV_p61       YRHVKRVGLT QDELTILSNI EFDVAEMCCE REVALQARRA QR....GEKP
CTV_p61       YKRVPDGAVT ADELESLRQL RSSVDVMCKD R.VSITPPPF NRLRRGSSRT
LIYV_P59      FKGLDMGKLT DEERLSIQTL RCITEDRS.E GTLATHNDLN SWILRP....
GLRaV3_p55    ITSKVRNTIN TADHASIIHY IKTNENQVTG TTLPHQL*.. ..........

CONSENSUS     y--------t -de--s---- ----e----e ---------- -r--------

BYV_p61       FQGWKGTKNE ISPHARSSIR VKKNNDSLLN ILWKDVGARS QRRLNPLHRK
CTV_p61       FRGR.GARGA SSRHMSRDVA TSGFNLPYHG RLYSTS*... ..........
LIYV_P59      .......... .......... .......... .......... ..........
GLRaV3_p55    .......... .......... .......... .......... ..........

CONSENSUS     ---------- ---------- ---------- ---------- ----------

BYV_p61       H*
CTV_p61       ..
LIYV_P59      ..
GLRaV3_p55    ..

CONSENSUS     --
```

TABLE 14

Nucleotide and Deduced Amino Acid Sequences of the Partial HSP90-Related Gene of GLRaV-3 Adapted for Cloning into Plant Expression Vectors.



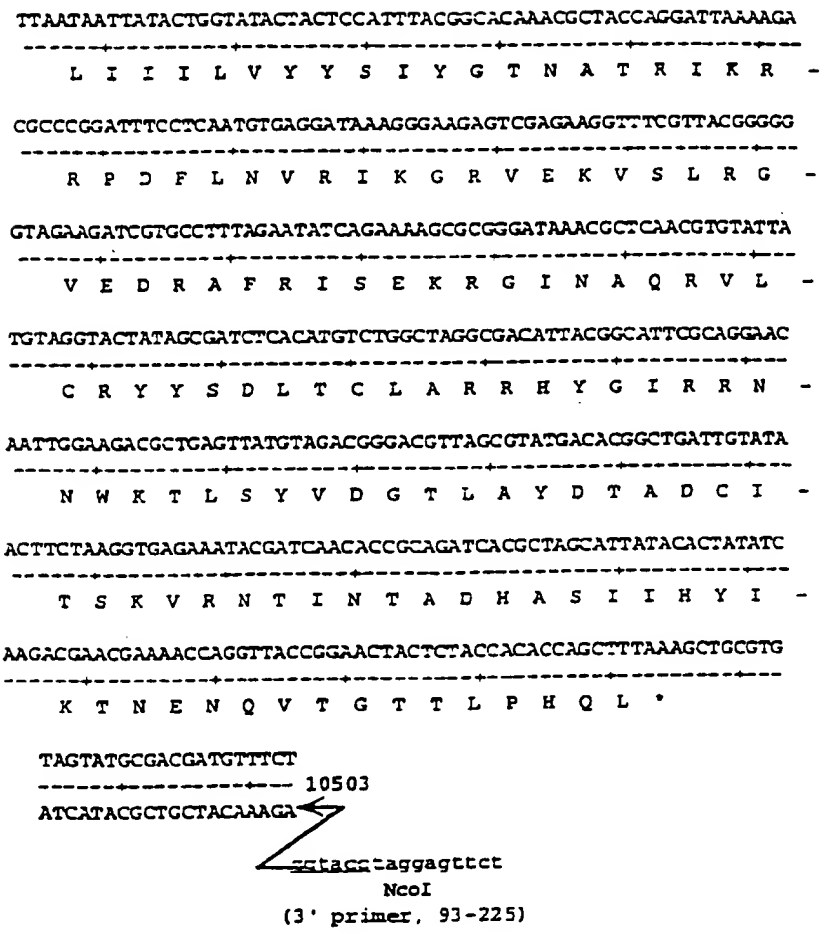
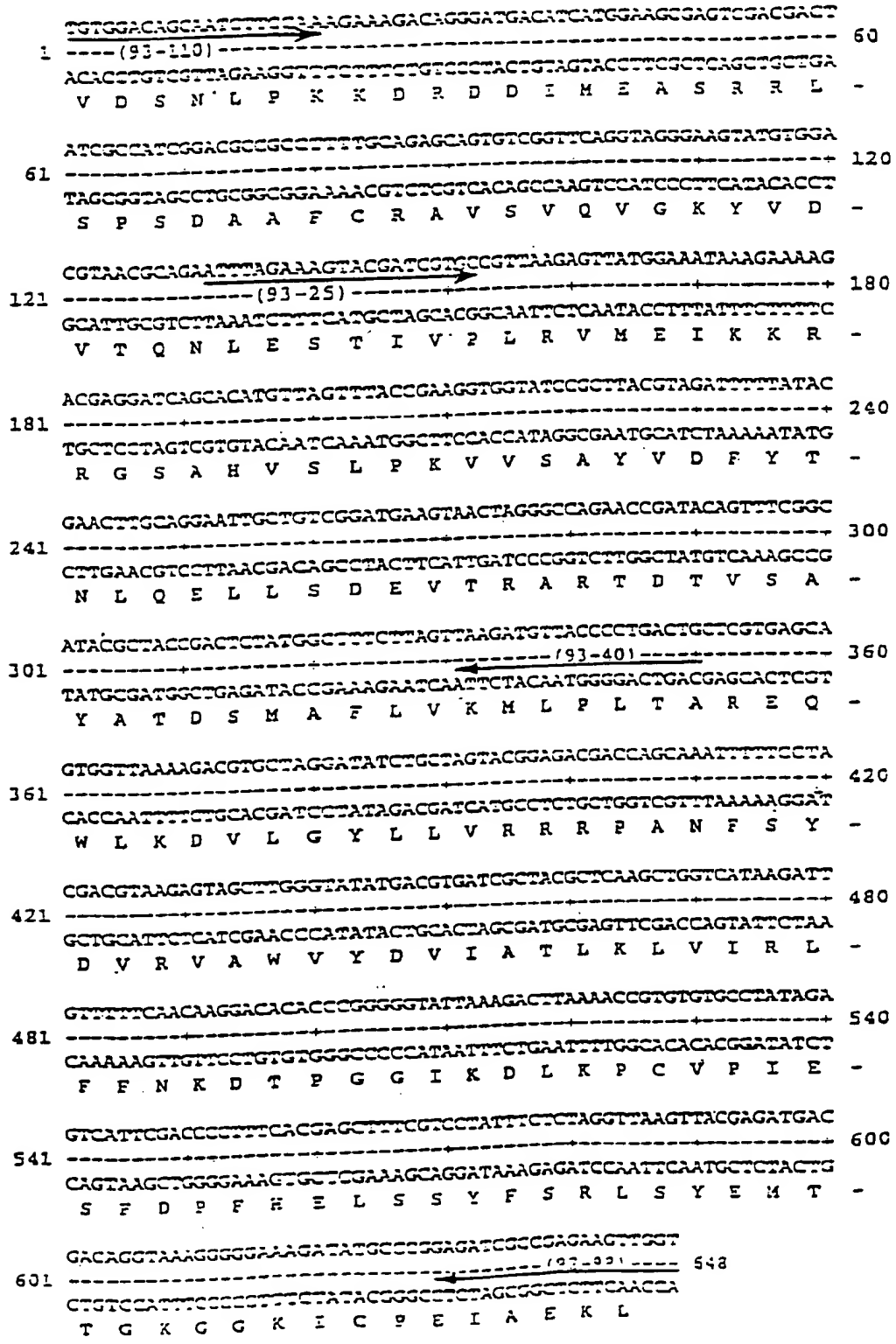

TTAATAATTATACTGGTATACTACTCCATTTACGGCACAAACGCTACCAGGATTAAAAGA
L I I I L V Y Y S I Y G T N A T R I K R -
CGCCCGGATTTCCTCAATGTGAGGATAAAGGGAAGAGTCGAGAAGGTTTCGTTACGGGGG
R P D F L N V R I K G R V E K V S L R G -
GTAGAAGATCGTGCCTTTAGAATATCAGAAAAGCGCGGGATAAACGCTCAACGTGTATTA
V E D R A F R I S E K R G I N A Q R V L -
TGTAGGTACTATAGCGATCTCACATGTCTGGCTAGGCGACATTACGGCATTCGCAGGAAC
C R Y Y S D L T C L A R R H Y G I R R N -
AATTGGAAGACGCTGAGTTATGTAGACGGGACGTTAGCGTATGACACGGCTGATTGTATA
N W K T L S Y V D G T L A Y D T A D C I -
ACTTCTAAGGTGAGAAATACGATCAACACCGCAGATCACGCTAGCATTATACACTATATC
T S K V R N T I N T A D H A S I I H Y I -
AAGACGAACGAAAACCAGGTTACCGGAACTACTCTACCACACCAGCTTTAAAGCTGCGTG
K T N E N Q V T G T T L P H Q L *
TAGTATGCGACGATGTTTCT
10503
ATCATACGCTGCTACAAAGA
ccatggtaggagtttct
NcoI
(3' primer, 93-225)

TABLE 15

Nucleotide and Deduced Amino Acid Sequences of a PCR-amplified Fragment of the GLRaV-3 Genome External and Internal Primes are underlined and their orientations are indicated by arrows.



CLAIMS:

1. An isolated DNA molecule encoding a protein or polypeptide of a grapevine leafroll virus.

2. An isolated DNA molecule according to claim 1, wherein the protein or polypeptide is selected from a group consisting of a helicase, an RNA-dependent RNA polymerase, an hsp70-related, an hsp90-related, a coat protein or polypeptide, coat protein repeat, ORF8 (p21), ORF9 (P20), ORF10 (P20) and ORF11 (p7).

3. An isolated DNA molecule according to claim 2, wherein the protein or polypeptide is a helicase having a molecular weight of from about 146 to about 151 kDa.

4. An isolated DNA molecule according to claim 3, wherein the protein or polypeptide comprises an amino acid sequence corresponding to SEQ ID NO:2.

5. An isolated DNA molecule according to claim 4, wherein the DNA molecule comprises a nucleotide sequence corresponding to SEQ ID NO:1.

6. An isolated DNA molecule according to claim 2, wherein the protein or polypeptide is an RNA-dependent RNA polymerase having a molecular weight of from about 59 to about 63 kDa.

7. An isolated DNA molecule according to claim 6, wherein the protein or polypeptide comprises an amino acid sequence corresponding to SEQ ID NO:4.

8. An isolated DNA molecule according to claim 7, wherein the DNA molecule comprises a nucleotide sequence corresponding to SEQ ID NO:3.

9. An isolated DNA molecule according to claim 2, wherein the protein or polypeptide is an hsp70-related protein or polypeptide having a molecular weight of from about 57 to about 61 kDa.

10. An isolated DNA molecule according to claim 9, wherein the protein or polypeptide comprises an amino acid sequence corresponding to SEQ ID NO:6.

11. An isolated DNA molecule according to claim 10, wherein the DNA molecule comprises a nucleotide sequence corresponding to SEQ ID NO:5.

12. An isolated DNA molecule according to claim 2, wherein the protein or polypeptide is an hsp90-related protein or polypeptide having a molecular weight of from about 53 to about 57 kDa.

13. An isolated DNA molecule according to claim 12, wherein the protein or polypeptide comprises an amino acid sequence corresponding to SEQ ID NO:8.

14. An isolated DNA molecule according to claim 13, wherein the DNA molecule comprises a nucleotide sequence corresponding to SEQ ID NO:7.

15. An isolated DNA molecule according to claim 2, wherein the protein or polypeptide is a coat protein or polypeptide having a molecular weight of from about 33 to about 43 kDa.

16. An isolated DNA molecule according to claim 15, wherein the protein or polypeptide comprises an amino acid sequence corresponding to SEQ ID NO:10.

17. An isolated DNA molecule according to claim 16, wherein the DNA molecule comprises a nucleotide sequence corresponding to SEQ ID NO:9.

18. An isolated DNA molecule according to claim 1, wherein the protein or polypeptide comprises an amino acid sequence corresponding to SEQ ID NO:12.

19. An isolated DNA molecule according to claim 18, wherein the DNA molecule comprises a nucleotide sequence corresponding to SEQ ID NO:11.

20. An isolated DNA molecule according to claim 1, wherein the protein or polypeptide comprises an amino acid sequence corresponding to SEQ ID NO:14.

21. An isolated DNA molecule according to claim 20, wherein the DNA molecule comprises a nucleotide sequence corresponding to SEQ ID NO:13.

22. An isolated DNA molecule according to claim 1, wherein the protein or polypeptide comprises an amino acid sequence corresponding to SEQ ID NO:16.

23. An isolated DNA molecule according to claim 22, wherein the DNA molecule comprises a nucleotide sequence corresponding to SEQ ID NO:15.

24. An isolated DNA molecule according to claim 1, wherein the protein or polypeptide comprises an amino acid sequence corresponding to SEQ ID NO:18.

25. An isolated DNA molecule according to claim 24, wherein the DNA molecule comprises a nucleotide sequence corresponding to SEQ ID NO:17.

26. An expression system comprising a DNA segment corresponding to a DNA molecule according to any of claims 1 to 25 in a vector heterologous to the DNA molecule and wherein a nucleotide sequence encoding a protein or polypeptide of a grapevine leafroll virus is operatively linked to nucleotide sequences which direct the expression of said protein or polypeptide.

27. A host cell transformed with a heterologous expression system according to claim 26.

28. A host cell according to claim 27, wherein the host cell is selected from the group consisting of *Agrobacterium vitis* and *Agrobacterium tumefaciens*.

29. A host cell according to claim 28, wherein the host cell is a grape cell or a citrus cell.

30. A transgenic *Vitis* scion cultivar or rootstock cultivar comprising a DNA sequence encoding a protein or a polypeptide of a grapevine leafroll virus according to any of claims 1-25.

31. A transgenic *Vitis* scion cultivar or rootstock cultivar according to claim 30, wherein the *Vitis* scion or rootstock cultivar is a scion cultivar selected from the group consisting of *Vitis vinifra*, Alden, Almeria, Anab-E-Shahi, Autumn Black, Beauty Seedless, Black Corinth, Black Damascus, Black Malvoisie, Black Prince, Blackrose, Bronx Seedless, Burgrave, Calmeria, Campbell Early, Canner, Cardinal, Catawba, Christmas, Concord, Dattier, Delight, Diamond, Dizmar, Duchess, Early Muscat, Emerald Seedless, Emperor, Exotic, Ferdinand de Lesseps, Fiesta, Flame seedless, Flame Tokay, Gasconade, Gold, Himrod, Hunisa, Hussiene, Isabella, Italia, July Muscat, Khandahar, Katta, Kourgane, Kishmishi, Loose Perlette, Malaga, Monukka, Muscat of Alexandria, Muscat Flame,

Muscat Hamburg, New York Muscat, Niabell, Niagara, Olivette blanche, Ontario, Pierce, Queen, Red Malaga, Ribier, Rish Baba, Romulus, Ruby Seedless, Schuyler, Seneca, Suavis (IP 365), Thompson seedless, Aleatico, Alicante Bouschet, Aligote, Alvarelhao, Aramon, Baco blanc (22A), Burger, Cabernet franc, Cabernet, Sauvignon, Calzin, Carignane, Charbono, Chardonnay, Chasselas dore, Chenin blanc, Clairette blanche, Early Burgundy, Emerald Riesling, Feher Szagos, Fernao Pires, Flora, French Colombard, Fresia, Furmint, Gamay, Gewurztraminer, Grand noir, Gray Riesling, Green Hungarian, Green Veltliner, Grenache, Grillo, Helena, Inzolia, Lagrein, Lambrusco de Salamino, Malbec, Malvasia bianca, Mataro, Melon, Merlot, Meunier, Mission, Montua de Pilas, Muscadelle du Bordelais, Muscat blanc, Muscat Ottonel, Muscat Saint-Vallier, Nebbiolo, Nebbiolo fino, Nebbiolo Lampia, Orange Muscat, Palomino, Pedro Ximenes, Petit Bouschet, Petite Sirah, Peverella, Pinot noir, Pinot Saint-George, Primitivo di Gioa, Red Veltliner, Refosco, Rkatsiteli, Royalty, Rubired, Ruby Cabernet, Saint-Emilion, Saint Macaire, Salvador, Sangiovese, Sauvignon blanc, Sauvignon gris, Sauvignon vert, Scarlet, Seibel 5279, Seibel 9110, Seibel 13053, Semillon, Servant, Shiraz, Souzao, Sultana Crimson, Sylvaner, Tannat, Teroldico, Tinta Madeira, Tinto cao, Touriga, Traminer, Trebbiano Toscano, Trousseau, Valdepenas, Viognier, Walschriesling, White Riesling, and Zinfandel.

32. A transgenic *Vitis* scion cultivar or rootstock cultivar according to claim 30, wherein the *Vitis* scion or rootstock cultivar is a rootstock cultivar selected from the group consisting of Couderc 1202, Couderc 1613, Couderc 1616, Couderc 3309, Dog Ridge, Foex 33 EM, Freedom, Ganzin 1 (A x R #1), Harmony, Kober 5BB, LN33, Millardet & de Grasset 41B, Millardet & de Grasset 420A, Millardet & de Grasset 101-14, Oppenheim 4 (SO4), Paulsen 775, Paulsen 1045, Paulsen 1103, Richter 99, Richter 110, Riparia Gloire, Ruggeri 225, Saint-George, Salt Creek, Teleki 5A, Vitis rupestris Constantia, *Vitis california*, and *Vitis girdiana*.

33. A method of imparting grapevine leafroll virus resistance to a *Vitis* scion cultivar or rootstock cultivar comprising:

transforming a *Vitis* scion cultivar or rootstock cultivar with a an expression system according to claim 26 wherein said vector is a plant transformation vector.

34. A method according to claim 33, wherein the *Vitis* scion cultivar or rootstock cultivar is a scion cultivar selected from the group consisting of *Vitis vinifra*, Alden, Almeria, Anab-E-Shahi, Autumn Black, Beauty Seedless, Black Corinth, Black Damascus, Black Malvoisie, Black Prince, Blackrose, Bronx Seedless, Burgrave, Calmeria, Campbell Early, Canner, Cardinal, Catawba, Christmas, Concord, Dattier, Delight, Diamond, Dizmar, Duchess, Early Muscat, Emerald Seedless, Emperor, Exotic, Ferdinand de Lesseps, Fiesta, Flame seedless, Flame Tokay, Gasconade, Gold, Himrod, Hunisa, Hussiene, Isabella, Italia, July Muscat, Khandahar, Katta, Kourgane, Kishmishi, Loose Perlette, Malaga, Monukka, Muscat of Alexandria, Muscat Flame, Muscat Hamburg, New York Muscat, Niabell, Niagara, Olivette blanche, Ontario, Pierce, Queen, Red Malaga, Ribier, Rish Baba, Romulus, Ruby Seedless, Schuyler, Seneca, Suavis (IP 365), Thompson seedless, Aleatico, Alicante Bouschet, Aligote, Alvarelhao, Aramon, Baco blanc (22A), Burger, Cabernet franc, Cabernet, Sauvignon, Calzin, Carignane, Charbono, Chardonnay, Chasselas dore, Chenin blanc, Clairette blanche, Early Burgundy, Emerald Riesling, Feher Szagos, Fernao Pires, Flora, French Colombard, Fresia, Furmint, Gamay, Gewurztraminer, Grand noir, Gray Riesling, Green Hungarian, Green Veltliner, Grenache, Grillo, Helena, Inzolia, Lagrein, Lambrusco de Salamino, Malbec, Malvasia bianca, Mataro, Melon, Merlot, Meunier, Mission, Montua de Pilas, Muscadelle du Bordelais, Muscat blanc, Muscat Ottonel, Muscat Saint-Vallier, Nebbiolo, Nebbiolo fino, Nebbiolo Lampia, Orange Muscat, Palomino, Pedro Ximenes, Petit Bouschet, Petite Sirah, Peverella, Pinot noir, Pinot Saint-

George, Primitivo di Gioa, Red Veltliner, Refosco, Rkatsiteli, Royalty, Rubired, Ruby Cabernet, Saint-Emilion, Saint Macaire, Salvador, Sangiovese, Sauvignon blanc, Sauvignon gris, Sauvignon vert, Scarlet, Seibel 5279, Seibel 9110, Seibel 13053, Semillon, Servant, Shiraz, Souzao, Sultana Crimson, Sylvaner, Tannat, Teroldico, Tinta Madeira, Tinto cao, Touriga, Traminer, Trebbiano Toscano, Trousseau, Valdepenas, Viognier, Walschriesling, White Riesling, and Zinfandel.

35. A method according to claim 33, wherein the *Vitis* scion cultivar or rootstock cultivar is a rootstock cultivar selected from the group consisting of Couderc 1202, Couderc 1613, Couderc 1616, Couderc 3309, Dog Ridge, Foex 33 EM, Freedom, Ganzin 1 (A x R #1), Harmony, Kober 5BB, LN33, Millardet & de Grasset 41B, Millardet & de Grasset 420A, Millardet & de Grasset 101-14, Oppenheim 4 (SO4), Paulsen 775, Paulsen 1045, Paulsen 1103, Richter 99, Richter 110, Riparia Gloire, Ruggeri 225, Saint-George, Salt Creek, Teleki 5A, Vitis rupestris Constantia, *Vitis californica*, and *Vitis girdiana*.

36. A method according to claim 33, wherein the grapevine leafroll virus is selected from the group consisting of GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5, and GLRaV-6.

37. A method according to claim 33, wherein said transforming is *Agrobacterium*-mediated.

38. A method according to claim 37, wherein said transforming comprises:

contacting tissue of the *Vitis* scion cultivar or rootstock cultivar with an inoculum of bacterium of the genus *Agrobacterium* transformed with an expression construct according to claims 26 and regenerating a transformed plant.

39. A method according to claim 38, wherein the tissue is selected from the group consisting of leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

40. A method according to claim 33, wherein said transforming comprises:

propelling particles at grape plant cells under conditions effective for the particles to penetrate into the cell interior and

introducing an expression vector comprising the DNA molecule into the cell interior.

41. A method according to claim 40, wherein the vector is associated with the particles, whereby the vector is carried into the cell interior together with the particles.

42. A method according to claim 41, wherein the vector surrounds the cell and is drawn into the cell by the particle's wake.

43. A transgenic citrus scion cultivar or rootstock cultivar transformed with an expression system according to claim 26, wherein said vector is a plant transformation vector.

44. A transgenic citrus scion cultivar or rootstock cultivar according to claim 43, wherein the citrus scion cultivar or rootstock cultivar is selected from the group consisting of lemon, lime, orange, grapefruit, pineapple, and tangerine.

45. A transgenic citrus scion cultivar or rootstock cultivar according to claim 43, wherein the citrus scion cultivar or rootstock cultivar is selected from the group consisting of Joppa, Maltaise Ovale, Parson (Parson Brown), Pera, Pineapple, Queen, Shamouti, Valencia, Tenerife, Imperial Doblefina, Washington Sanguine, Moro, Sanguinello Moscato,

Spanish Sanguinelli, Tarocco, Atwood, Australian, Bahia, Baiana, Cram, Dalmau, Eddy, Fisher, Frost Washington, Gillette, LengNavelina, Washington, Satsuma Mandarin, Dancy, Robinson, Ponkan, Duncan, Marsh, Pink Marsh, Ruby Red, Red Seedless, Smooth Seville, Orlando Tangelo, Eureka, Lisbon, Meyer Lemon', Rough Lemon, Sour Orange, Persian Lime, West Indian Lime, Bearss, Sweet Lime, Troyer Citrange, and Citrus trifoliata.

46. A method for producing a virus-resistant citrus scion cultivar or rootstock cultivar comprising:

transforming citrus tissue with an expression vector according to claim 26.

47. A method according to claim 46, wherein said transforming is *Agrobacterium*-mediated.

48. A method according to claim 46, wherein the tissue is selected from the group consisting of leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

49. The method of claim 46 wherein said expression vector is introduced into plant cells by a biolistic method.

50. An antibody or binding portion thereof or probe recognizing the protein or polypeptide encoded by the DNA molecule according to any of claims 1 to 25.

51. An isolated protein or polypeptide corresponding to a protein or polypeptide of a grapevine leafroll virus encoded by a DNA molecule according to any of claims 1-25.

52. A method for detection of grapevine leafroll virus in a sample, said method comprising:

providing an antibody or binding portion thereof recognizing the encoded protein or polypeptide according to any of claims 1 to 25;

contacting the sample with the antibody or binding portion thereof; and

detecting any reaction which indicates that grapevine leafroll virus is present in the sample using an assay system.

53. A method according to claim 55, wherein the assay system is selected from the group consisting of an enzyme-linked immunosorbent assay, a radioimmunoassay, a gel diffusion precipitin reaction assay, an immunodiffusion assay, an agglutination assay, a fluorescent immunoassay, a protein A immunoassay, and an immunoelectrophoresis assay.

54. A method for detection of grapevine leafroll virus in a sample, said method comprising:

providing a nucleotide sequence of the DNA molecule according to any of claims 1 to 25 as a probe in a nucleic acid hybridization assay;

contacting the sample with the probe; and

detecting any reaction which indicates that grapevine leafroll virus is present in the sample.

55. A method for detection of grapevine leafroll virus in a sample:

providing a nucleotide sequence of the DNA molecule according to any of claims 1 to 25 as a probe in a gene amplification detection procedure;

contacting the sample with the probe; and

detecting any reaction which indicates that grapevine leafroll virus is present in the sample.

AN 1996:479806 CAPLUS
DN 125:138016
TI Coat protein gene identification, genome organization, and pcr detection of grapevine leafroll associated closterovirus-3 and study towards transgenic grapevines (vitis)
AU Ling, Kai-Shu
CS Cornell Univ., Ithaca, NY, USA
SO (1995) 232 pp. Avail.: Univ. Microfilms Int., Order No. DA9624860 From: Diss. Abstr. Int., B 1996, 57(3), 1539
DT Dissertation
LA English
CC 11-5 (Plant Biochemistry)
AB Unavailable
ST closterovirus grapevine leafroll gene coat **protein**; genome organization **grapevine leafroll virus**
IT Genome
Grape
(coat protein gene identification, genome organization, and pcr detection of grapevine leafroll assocd. closterovirus-3 and study towards transgenic grapevines)
IT Gene, plant
RL: BOC (Biological occurrence); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(coat protein gene identification, genome organization, and pcr detection of grapevine leafroll assocd. closterovirus-3 and study towards transgenic grapevines)
IT Virus, plant
(clostero-, coat protein gene identification, genome organization, and pcr detection of grapevine leafroll assocd. closterovirus-3 and study towards transgenic grapevines)
IT Proteins, specific or class
RL: BOC (Biological occurrence); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(coat, coat protein gene identification, genome organization, and pcr detection of grapevine leafroll assocd. closterovirus-3 and study towards transgenic grapevines)
IT Virus, plant
(leafroll, coat protein gene identification, genome organization, and pcr detection of grapevine leafroll assocd. closterovirus-3 and study towards transgenic grapevines)